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# Effects of Different Feeding Frequency on the Growth, Feed utilization and Body Composition of Juvenile Eel *Anguilla japonica* in Semi-RAS(Recirculating Aquaculture System)

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# 반순환여과시스템내에서 사료 공급 횟수가 뱀장어(*Anguilla japonica*) 치어의 성장, 사료 이용성 및 체조성에 미치는 영향

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**Abstract**

A feeding trial was conducted to investigate the effect of feeding frequency on the growth performance and body composition of juvenile eel, *Anguilla japonica*. Duplicate groups of fish (initial fish weight, 0.93 g/fish) were fed to apparent satiation at one, two, three, or four meals per day for 8 weeks. The results of the present study showed that weight gain and specific growth rate of fish fed one meal per day were significantly lower than those of fish fed three and four meals per day, were not affected by two meals per day. Daily feed intake of fish fed one meal per day was significantly lower than that of fish fed four meals per day, was not affected by two meals and three meals per day. Feed efficiency, daily protein intake and protein efficiency ratio were not affected by feeding frequency. The moisture, crude protein, crude lipid and crude ash were not affected by feeding frequency. Consequently, the present results suggested that optimum feeding frequency of juvenile eel (average weigth 0.9 to 3 g) is three meals per day.

**Key words : Eel, *Anguilla japonica*, Feeding frequency, Growth, Feed utilization**

## Ⅰ. Introduction

There are 18 species of fish in the genus Anguillidae that are known worldwide (Aoyama et al., 2001), and the species *Anguilla japonica* (Japanese eel) and *Anguilla marmorata* (Marbled eel) inhabit South Korea (Kim et al., 2008). Four species of eels are commonly farmed: *Anguilla anguilla* (North American eel), *Anguilla rostrata* (European eel), *Anguilla bicolor* (Southeast Asian eel), and *Anguilla japonica* (Japanese eel), which are mainly farmed species in South Korea (Kim YH, 2013). *Anguilla japonica* is a representative freshwater fish species that is rich in protein, fat, minerals, and vitamins compared to other fish species and has long been used as a favorite food in Southeast Asia, including South Korea, Japan, and China (Choi et al., 2011). It is a very

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important cultured species in East Asia owing to its high market value, desirable taste, and recent supply shortage (Zheng et al., 2019). Regarding the history of eel farming in South Korea, until the early 1970s, live eels floating on the coast were mainly captured, grown to an average weight of about 2 g, and exported to Japan and Taiwan as intermediate nursery stock. Domestic farming has been practiced since the late 1970s, and since the 2000s, farming has been actively carried out with the introduction of a practical recirculating aquaculture system. Therefore, to increase the production and productivity of eel farming, various studies are needed on breeding methods, feed quality improvement, and feeding supply systems. Feeding methods that can increase the growth rate and survival of fish, reduce size differences among individuals, and minimize the amount of wasted feed and labor costs, along with maintaining feed efficiency, are necessary (Kubitz and Lovshin, 1999; Oh and Park, 2016; Kim YO, 2022a). In particular, feeding frequency is an important factor affecting feed availability, growth, and metabolite excretion in fish (Silva et al., 2007; Biswas et al., 2010), and inappropriate feeding frequency reduces fish growth and feed efficiency, which ultimately increases the cost of fish aquaculture production (Oh and Maran, 2015; Kim et al., 2020). Thus, information on the optimal number of feedings that are economically feasible is essential for successful fish farming (Silva et al., 2007; Kim YO, 2022b). In this regard, feeding frequency plays a crucial role in improving aquaculture productivity by inducing maximum growth and feed efficiency of farmed fish; therefore, determining the optimal feeding frequency is necessary (Kim et al., 2022a; Kim et al., 2022b). In addition, proper feeding frequency can provide information on the daily feed intake of farmed fish and the timing of feeding to establish a planned feeding system (Oh and Park, 2016).

Therefore, to increase the production of eel aquaculture, the appropriate feeding frequency should be investigated and applied to aquaculture sites. This study was conducted to investigate the effects of feeding frequency on growth and feed utilization and composition in eel fry rearing.

# Ⅱ. Material and methods

## 1. Fish and rearing condition

Self-reared juvenile eel, *Anguilla japonica,* from the Chungcheongbuk-do Island Fisheries Research Institute, was used in the experiment. Two weeks before the start of the experiment, experimental feed was supplied twice a day for pre-breeding. After the pre-breeding period, 30 juvenile eels (0.93 ± 0.01 g) were randomly distribute to 8 tanks (200 L each) with a replicate groups and reared for 8 weeks.

Eel breeding was conducted similar to a previous experiment (Kim YO, 2022a; Kim YO, 2022b) in a semi-circular filtration system comprising one set of square-shaped sedimentation tanks (2 m × 1 m × 1.2 m; 2,000 L) and eight circular experimental tanks (diameter 0.6 m × height 1 m; 200 L), with water temperature maintained at 26 ℃. The circular water tanks containing the experimental fish were maintained at the same breeding environment with water temperature of 25 ℃, pH of 6.2–7.8, and DO of 6.3–7.5.

## 2. Experimental design

The feed supplied during the experiment was the commercial formulated feed (Chunhajeil Co., Daejeon, Korea; 8.4% moisture, 56.1% crude protein, 11.0% crude lipid, and 8.2% crude ash) mainly used by fish farmers on site. Feed was supplied four times (08:30, 11:30, 14:30, 17:30) a day, thrice (08:30, 13:00, 17:30) a day, twice (08:30, 17:30) a day, and once (08:30) a day full stomach.

### 3. Fish measurement and body content analysis

For fish measurement, fish were not fed for 1 day before the day of measurement, at the beginning and at the end of the rearing experiment. Length and weight of the fish were measured under anesthesia using a 100 ppm aqueous solution of tricaine methanesulfonate (MS 222, Sigma, St. Louis, MO, USA).

To analyze body composition, ten fish from each experimental tank were collected and frozen at –25 °C before analysis. Experimental feed and general whole-body proximate were analyzed using standard procedures (AOAC, 1995). Crude protein content was measured by the Kjeldahl method using an Auto Kjeldahl System (Buchi B-324/435/412, Switzerland; Metrohm 8-719/806, Switzerland). Crude lipid was extracted using ether, and moisture was measured after drying in a dry oven at 105 °C for 6 h. Crude ash content was measured after burning in a muffle furnace at 600 °C for 4 h.

### 4. Statistical analysis

For statistical analysis of results, one-way ANOVA was performed by using SPSS Ver. 20 (SPSS Inc., Chicago, IL, U.S.A.), followed by analysis of different between mean values using Duncan’s multiple range test (P<0.05) (Duncan’s, 1995). Levene’s test was used to validate the homogeneity of variance, and percentage data were arcsine-transformed prior to ANOVA.

<Table 1> Ingredient and proximate composition of experimental diets for eel *Anguilla japonica.*

| Ingredients (%) | Diets |
|---|---|
| Commercial diet[1] | |
| Chemical analysis (% of dry matter basis) | |
| Moisture | 8.4 |
| Crude protein | 56.1 |
| Crude lipid | 11.0 |
| Crude ash | 8.2 |

[1]Fish Commercial formulated feed produced from Chunhajeil incorporation (Daejeon, Korea).

## Ⅲ. Results

Growth and feed utilization of eel juveniles as a function of feeding frequency are shown in <Tables 2 and 3>, respectively. The survival rate of all experimental groups during the rearing experiment was 100%, with no significant difference between experimental groups (P>0.05). Weight gain and specific growth rate were significantly lower in the one meal a day feeding group than in the three meals a day and four meals a day feeding groups (P<0.05), with no significant difference observed in the two meals a day feeding group (P>0.05). Feed efficiency was not significantly different between treatments (P>0.05). Daily feed intake was significantly lower in the one meal a day group than in the four meals a day group (P<0.05), with no significant difference between the two meals a day and three meals a day groups (P>0.05). Daily protein intake and protein efficiency ratio were not significantly different between the experimental groups (P>0.05).

The condition factor and coefficient of variation of eel juveniles fed the experimental diets according to the number of feedings for 8 weeks are shown in <Table 4>. Condition factor (CF), coefficient of variation of body length (CVBL), and coefficient of variation of body weight (CVBW) were not significantly different between the experimental groups (P>0.05).

The condition factor and coefficient of variation of eel juveniles fed the experimental diets according to the number of feedings for 8 weeks are shown in Table 4. Condition factor (CF), coefficient of variation of body length (CVBL), and coefficient of variation of body weight (CVBW) were not significantly different between the experimental groups (P>0.05).

<Table 2> Growth performance of eel *Anguilla japonica* fed experiment diets for 8 weeks[1]

| Feeding frequency /day | Initial mean weight (g) | Final mean weight (g) | Survival (%) | Weight gain (%)[2] | Specific growth rate (%/day)[3] |
|---|---|---|---|---|---|
| One meal | 0.94±0.02$^{ns}$ | 2.59±0.04$^{a}$ | 100±0.0$^{ns}$ | 176.9±0.25$^{a}$ | 1.82±0.01$^{a}$ |
| Two meals | 0.92±0.01 | 2.75±0.04$^{ab}$ | 100±0.0 | 198.4±0.55$^{ab}$ | 1.96±0.01$^{ab}$ |
| Three meals | 0.93±0.02 | 3.07±0.13$^{b}$ | 100±0.0 | 232.9±20.7$^{b}$ | 2.15±0.12$^{b}$ |
| Four meals | 0.93±0.01 | 3.09±0.19$^{b}$ | 100±0.0 | 232.4±15.5$^{b}$ | 2.15±0.09$^{b}$ |

[1]Values (mean±SE of duplicate groups) with different superscripts in the same column are significantly different (P<0.05).
[2]Weight gain (%) = (final body weight - initial body weight) × 100/initial body weight.
[3]Specific growth rate = (Ln final weight of fish − Ln initial weight of fish) × 100/days of feeding trial.
$^{ns}$Not significant (P>0.05).

<Table 3> Daily feed intake (DFI), feed efficiency (FE), daily protein intake (DPI) and protein efficiency ratio (PER) of eel *Anguilla japonica* fed experiment diets for 8 weeks[1]

| Feeding frequency /day | DFI(%)[2] | FE(%)[3] | DPI(%)[4] | PER(%)[5] |
|---|---|---|---|---|
| One meal | 2.13±0.05$^{a}$ | 78.9±1.80$^{ns}$ | 1.17±0.03$^{ns}$ | 1.44±0.04$^{ns}$ |
| Two meals | 2.26±0.06$^{ab}$ | 78.9±2.05 | 1.24±0.03 | 1.44±0.04 |
| Three meals | 2.37±0.06$^{ab}$ | 81.0±1.45 | 1.30±0.03 | 1.47±0.03 |
| Four meals | 2.56±0.19$^{b}$ | 75.7±7.80 | 1.41±0.11 | 1.38±0.15 |

[1]Values (mean±SE of duplicate groups) with different superscripts in the same column are significantly different (P<0.05).
[2]Daily feed intake = feed intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.) × days reared / 2].
[3]Feed efficiency = fish wet weight gain×100/feed intake (dry matter).
[4]Daily protein intake = protein intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.) × days reared / 2].
[5]Protein efficiency ratio = weight gain of fish / protein intake.
$^{ns}$Not significant (P>0.05).

<Table 4> Condition factor (CF), coefficient variation of body length (CVBL), and body weight (CVBW) of eel *Anguilla japonica* fed experiment diets for 8 weeks[1]

| Feeding frequency / day | CF(%)[2] | CVBL(%)[3] | CVBW(%)[4] |
|---|---|---|---|
| One meal | 0.09±0.01[ns] | 13.5±0.55[ns] | 56.4±1.05[ns] |
| Two meals | 0.10±0.01 | 13.3±0.35 | 51.2±1.75 |
| Three meals | 0.10±0.01 | 11.7±0.40 | 54.8±1.55 |
| Four meals | 0.10±0.01 | 13.1±0.60 | 53.1±1.65 |

[1]Values (mean±SE of duplicate groups) with different superscripts in the same column are significantly different (P<0.05).
[2]CF(%) = [weight of fish / (length of fish)$^3$] × 100.
[3]CVBL(%) = (standard deviation of final length of fish / mean final length of fish) × 100.
[4]CVBW(%) = (standard deviation of final weight of fish / mean final weight of fish) × 100.
[ns]Not significant (P>0.05).

<Table 5> Proximate composition (%) of eel *Anguilla japonica* fed experiment diets for 8 weeks[1]

| | Diets | | | |
|---|---|---|---|---|
| | One meal | Two meals | Three meals | Four meals |
| Proximate composition (% wet weight) | | | | |
| Moisture | 65.0±1.80[ns] | 64.8±2.00 | 64.0±0.10 | 63.1±0.90 |
| Crude protein | 15.4±0.05[ns] | 15.6±0.45 | 16.4±0.35 | 16.2±0.01 |
| Crude lipid | 18.3±0.40[ns] | 18.5±1.25 | 19.6±0.10 | 19.9±0.80 |
| Crude ash | 1.18±0.12[ns] | 1.23±0.03 | 1.01±0.10 | 1.15±0.20 |

[1]Values (mean±SE of duplicate groups) with different superscripts in the same column are significantly different (P<0.05).
[ns]Not significant (P>0.05).

<Table 5> shows the results of the principal component analysis of the entire fish body according to the number of feedings. The moisture, crude protein, crude lipid, and crude ash contents of the entire fish body were not significantly different between the experimental groups (P>0.05).

## Ⅳ. Discussion

Determining the appropriate feeding frequency for farmed fish species is important for improving aquaculture productivity to obtain maximum growth and feed efficiency and for reducing economic loss and water pollution caused by feed loss due to overfeeding (Ng et al., 2000; Mihelakakis et al., 2002). The optimal feeding frequency has been reported to differ depending on factors, such as diet, habitat environment, and the size of the fish species. Studies that investigated the appropriate feeding frequency to induce maximum growth have reported that rainbow trout (*Oncorhynchus mykiss*) (Ruohonen et al., 1998) and koi (*Cyprinus carpio* var. koi) (Kim and Lee, 2010) are suitable for feeding four meals a day; tilapia (*Oreochromis niloticus*) (Riche et al., 2004), Pacific cod (*Gadus macrocephalus*) (Choi et al., 2011), and Crucian carp (*Carassius auratus*) (Kim Yo, 2022b) three meals a day; masu salmon (*Oncorhynchus masou*) (Seong and Kim, 2008), golden mandarin fish (*Siniperca scherzeri*) (Kim et al., 2020), and pond loach (*Misgurnus mizolepis*) (Kim YO, 2022a) two meals a day; black rockfish (*Sebastes schlegelii*) (Lee et al., 2000b) one meal a day; and esturary grouper (*Epinephelus tauvina*) one meal every 2 days (Chua and Teng, 1978). Although increasing the feeding frequency increases growth and feed intake of fish, many studies have reported that feeding more than a certain frequency does not affect the fish growth or actually reduces fish growth (Lee et al., 2000a; Mizanur and Bai, 2014; Oh and Maran 2015; Oh and Park, 2016; Kim et al., 2020; Kim YO, 2022a; Kim Yo, 2022b). Likewise, in this study, as the feeding frequency increased, weight gain, the daily growth rate, and the daily feed intake rate increased. As the feeding frequency increased, although the daily feed intake rate increased, there was no difference in the growth and daily growth rates between the experimental groups fed with three and four meals a day. A similar trend was also shown in other fish species.

In an experiment conducted on rainbow trout, Hong Kong grouper (*Epinephelus akaara*), olive flounder (*Paralichthys olivaceus*), golden mandarin fish, pond loach, and Crucian carp, it was reported that the growth rates did not improve further with more than the appropriate feeding frequencies (Grayton and Beamish, 1977; Kayano et al., 1993; Kim et al., 2009; Kim et al., 2020; Kim YO, 2022a; Kim Yo, 2022b). On the other hand, in an experiment targeting black rockfish and dolly varden char (*Salvelinus malma*) fry, the growth rate decreased at a certain feeding frequency in the results (Lee et al., 1999; Lee et al., 2013; Oh and Park, 2016; Guo et al., 2018). Depending on the species and size of the fish, the frequency and amount of feed intake may vary. In this study, The daily feed intake rate increased for groups fed up to four times a day as the feeding frequency increased, although there was no difference in the feed efficiency among the experimental groups even when the daily feed intake rate was increased according to the increase in feeding frequency. This is because when the feeding frequency is increased or the feeding interval is shortened, the time for the excessively ingested feed to pass through the digestive tract decreases, resulting in inefficient digestion (Biswas et al., 2010; Mizanur and Bai, 2014; Oh and Park, 2016; Guo et al., 2018). Regarding striped beakfish (*Oplegnathus fasciatus*) (Oh and Maran, 2015) and rohu (*Labeo rohita*) (Biswas et al., 2006), The feeding frequency appeared to have no effect on the feed efficiency, showing similar results to this experiment; however, it has also been shown that the feeding frequency has an effect on the feed efficiency (Kang et al., 2015; Kim et al., 2020; Kim YO, 2022a; Kim YO, 2022b). These differences were shown to vary depending on the fish species and experimental conditions.

In addition, even between the same fish species, the optimal feeding frequency for the maximum growth of fish varies depending on the size of the fish body, type and nutrient content of the feed, and breeding conditions. In particular, it varies depending on the size of the fish body; Regarding olive flounder, it was reported that the optimal feeding frequency was three meals a day at satiation for fry with an average weight of 1.5 to 4 g (Lee et al., 1999), two or three meals a day at satiation for fry with an average weight of 3.5 to 15 g (Lee et al., 2000a), and two meals a day for fry with an average weight of 45 to 53 g (Kim et al., 2005a). For eels, it was reported that the optimal feeding frequency for 6.8-mm fry was five meals a day (Kim et al., 2020). This is considered to be due to the fact that as the size of the body increases, the internal organs grow as well, taking longer time for the intestines to be emptied after feeding. In view of these results, it is thought to be reasonable to determine the appropriate feeding frequency considering the species and size of the target fish. It is judged that the optimal feeding frequency of eel fry (0.9~3 g) in this study is three meals a day.

As a result of this experiment, there was no difference in size (i.e., CV) between objects according to the feeding frequency, showing similar results to the previous gibel carp (*Carassius auratus gibelio*) (Zhou et al., 2003), white sturgeon (*Acipenser transmontanus*) (Cui et al., 1997), *Misgurnus mizolepis*) (Kim YO, 2022a), and *Carassius auratus* (Kim YO, 2022b) studies.

It has been reported that excessive feeding beyond the optimal feeding frequency can decrease fish quality by increasing the fat accumulated in the body (Yao et al., 1994; Oh and Maran 2015; Kim YO, 2022a; Kim YO, 2022b). However, in this experiment, no significant difference was found in moisture, the crude protein, crude lipid and crude ash content of whole fish in all the experiment groups regardless of the feeding frequency; similar reports exist (Lee et al., 1999; Kim et al., 2005a; Kim et al., 2005b; Lee et al., 2013; Kim et al., 2020). This is considered to be due to the energy ratio of protein of the experimental feed being optimal or due to having no difference in the feed intake rate when feeding more than two meals a day.

Based on the results, the optimal feeding frequency is three meals a day at satiation, considering the growth and feed availability of eel fry (average weight 0.9 to 3 g).

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