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Effects of Different Feeding Frequency on the Growth, Feed Utilization and Body Composition of Juvenile Mud Loach *Misgurnus mizolepis* in Semi-RAS(Recirculating Aquaculture System)

Yi-Oh KIM†

†Chungcheongbuk-do Inland Fisheries Research Institute(researcher)

반순환여과시스템내에서 사료공급 횟수가 미꾸라지(*Misgurnus mizolepis*) 치어의 성장, 사료이용 및 체조성에 미치는 영향

김 이 오†

†충청북도내수면산업연구소(연구사)

Abstract

A feeding trial was conducted to investigate the effect of feeding frequency on the growth performance and body composition of juvenile mud loach, *Misgurnus mizolepis*. Duplicate groups of fish (initial fish weight, 1.0 g/fish) were fed to apparent satiation at one, two, three, or four meals per day for 10 weeks. The results of the present study showed that weight gain of fish fed one meal per day was significantly ($P<0.05$) lower than those fish fed two, three and four meals per day. Feed efficiency of fish fed one meals per day was significantly ($P<0.05$) lower than other experimental groups. Consequently, we can conclude that the optimum feeding frequency of juvenile mud loach appeared two twice per day.

Key words : Mud loach, *Misgurnus mizolepis*, Feeding frequency, Growth, Feed utilization

Ⅰ. Introduction

The mud loach (*Misgurnus mizolepis*) is one of the commercially important freshwater endemic species in China and Korea (Lee and Kim, 2012). The mud loach has superior potential for inland fisheries due to adult body size, slender shape, faster growth and resistance to disease (Park et al., 2006). However, wild stocks of mud loach have been rapidly decreasing due to fishing and habitat destruction, whereas commercial demand has continued to increase. Therefore, it is necessary to increase production of this species through aquaculture (Park et al., 1994).

To increase yields in mud loach farming, various aspects of the system must be optimized, including rearing technology, extruded pellet quality, and the feed supply system. The feed intake of fish is affected by their rearing environment, the feed composition, the form of feed and the feed supply system (Lee et al., 2000a; Lee et al., 2000b), all of which should be examined to improve fish growth and feed efficiency (Lee et al., 2000b; Ng et al., 2000). In particular, feeding frequency plays an important role in feed intake, as well as growth and waste discharge of fish (Silva et al., 2007).

† Corresponding author : 043-220-6531, kimio@korea.kr

Inappropriate feeding frequency reduces fish growth and feeding efficiency, ultimately increase in production cost (Oh and Maran 2015; Lee et al., 2000a). Therefore, it is essential to use the optimum feeding frequency for successful fish farming (Silva et al., 2007; Oh and Maran 2015). The optimum feeding frequency for maximum growth of fish varies depending on fish species, size of fish, form of feed, feed composition and feeding rate (Wang et al., 1998). Inappropriate feeding frequency can hamper growth, cause high mortality of fish, require more labor, increase production costs, and degrade water quality (Kubitz and Lovshin, 1999), whereas the optimum feeding frequency improves growth and minimizes feed waste, consequently improving production efficiency (Xie et al., 2011),

The present study aimed to investigate the effects of feeding frequency on the growth, body composition of juvenile mud loach.

Ⅱ. Material and methods

1. Fish and rearing condition

Juvenile mud loach were acclimated to a diet of extruded pellets in the Chungcheongbuk-do Inland Fisheries Research Institute, two meals (09:00 and 17:00) a day for four weeks before the main experiment. The experimental diet are commercial extrude pellets (Chunhajeil Co., Daejeon, Korea: 24.8% moisture, 57.1% crude protein, 11.3% crude lipid and 8.4% ash). The experiment was conducted using a pilot-scaled semi-recirculation system consisting of a biofilter tank (volume 2,000 L) and 8 FRP round rearing tanks (200 L), based on a previous study (Kim, 2022). Clean water was continuously supplied at 5 L/min with continuous waste extraction and replenishment of dissolved oxygen. During the experimental period, water temperature, pH and dissolved oxygen were monitored daily. Water temperature was maintained at 24.0 ± 0.1℃, and the pH and dissolved oxygen concentration were 6.5-7.9 and 6.0-7.7 mg/L, respectively throughout the experiment.

2. Experimental design

After the acclimation period, 240 juvenile mud loach(mean weight 1.0 g) were randomly distributed to 8 tanks (200 L each) with 30 fish per tank. Each tank was assigned to one of four feeding groups: one, two, three, or four meals a day. The feeding times were set as follows: 08:30 for the group that received one meal a day; 08:30 and 17:30 for the group that received two meals a day; 08:30, 13:00, and 17:30 for the group that received three meals a day; and 08:30, 11:30, 14:30, and 17:30 for the group that received four meals a day. The experimental feed was the same as the feed used during the acclimation period, and juveniles were hand-fed satiety based on visual observation at the assigned times. The experiment lasted for 10 weeks.

3. Fish measurement and body content analysis

Fish were not fed for one day before the day of measurement both at the beginning and at the end of the rearing experiment. The fish were weighed at the beginning and end of the experiment under anesthesia using 100 mg/L tricaineme thanesulfonate (MS 222, Sigma, St. Louis, MO, USA) solution. The length and mass of each was measured at the end of the experiment; all fish were starved for 24 h prior to measuring.

For analysis of body composition, ten fish from each tank were randomly collected at the end of the experiment and stored at -25℃ before analysis. Chemical composition of the experimental diet and fish 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 by using ether, and moisture was measured after drying at 105℃ in a dry oven for 6 hr. Ash contents was measured after burning at 600℃ in a muffle furnace for 4 hr.

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 difference between mean values using Duncan's multiple range test ($P<0.05$) (Duncan, 1995). Levene's test was used to validate the homogeneity of variance, and percentage data were arcsine-transformed prior to ANOVA.

Ⅲ. Results

Growth and feed utilization of juvenile mud loach feeding frequencies were presented in Tables 2 and 3. Survival rates during the experiment period were 100% for all groups, with no significant differences between experimental groups ($P>0.05$). Weight gain (WG) and specific growth rate (SGR) were significantly ($P<0.05$) lower in the experimental group that received one meal a day than in that received two, three or four meals a day. However, there was no significant difference among the experimental groups that received two, three, or four meals a day. Daily feed intake (DFI) and daily protein intake (DPI) were significantly lower in the experimental group that received one meal and two meal a day than in that received three and four meals a day. Feed efficiency (FE) was significantly lower in the experimental group that received one meal a day than in that received two, three or four meals a day. However, there was no significant difference among the experimental groups that received two, three, or four meals a day. Protein efficiency ratio (PER) was significantly lowerr in the experimental group that received one meal and four meal a day than in that received two and three meals a day. Condition factor (CF), coefficient variation of body length (CVBL) and coefficient variation of body length (CVBW) of juvenile mud loach feeding frequencies were presented in Tables 4. Condition factor (CF), coefficient variation of body length (CVBL) and coefficient variation of body length (CVBW) were no significant difference among the experimental groups. Changes in whole body proximate composition of juvenile mud loach according to feeding frequency are presented in Table 5. There were no significant differences in the amounts of moisture, protein and ash contents of the whole body among all experimental groups. Lipid content was significantly higher in the experimental group that received four meals a day than in those that received one meals a day, but there was no significant difference among the experimental groups that received two, three, or four meals a day.

Ⅳ. Discussion

Feeding frequency had significant effects on WG, SGR, FE and PER of juvenile mud loach. Our data showed that two meals a day was the optimum feeding frequency for juvenile mud loach, producing a higher specific growth rate, feed efficiency, and protein efficiency ratio. However, growth was retarded when fish received three or four meals a day. Optimum feeding frequencies differ widely depending on fish species. In previous studies, optimum feeding frequencies were four meals a day for rainbow trout (Ruohonen et al., 1998) and fancy carp (Kim and Lee, 2010), three meals a day for sunfish (Wang et al., 1998) and pacific cod (Choi et al, 2011), two meals a day for yellowtail flounder (Dwyer et al., 2002) and mandarin fish (Kim et al., 2020), one meal a day for Korean rockfish (Lee et al., 2000b), and one meal every two days for esturary grouper (Chua and Teng, 1978). In the present study, growth and feed efficiency significantly lower one meal a day than in that received two, three or four meals a day. However, there was no significant difference among the experimental groups that received two, three, or four meals a day. Juvenile mud loach showed constant growth higher feeding frequencies (i.e., three or four meals a day) than the optimum feeding frequency (i.e., two meals a day), consistent with previous reports on red-spotted grouper (*Epinephelusakaara*), olive flounder and mandarin fish (Kayano et al., 1993; Kim et al., 2009; Kim et al., 2020). On the contrary, the growth rate of juvenile olive flounder and black rockfish were higher at certain feeding frequencies and declined these frequencies were exceeded (Lee et al., 1999; Lee et al, 2013). This is likely attributed to the decrease in digestibility as time was shortened for excessively ingested feed to pass through the digestive tract, even when feed intake increased due to the increased feeding frequency (Oh and Park, 2016; Kim et al., 2020). The appropriate feeding frequency or amount of feed intake may vary according to the fish type and size; in this study, daily feed intake increased up to three times a day but not beyond that. These results indicate that it does not increase any further for mud loaches once feeding frequency exceeds the optimum (Kayano et al., 1993; Lee et al., 2000b; Kim et al., 2020), which is consistent with the results of this study. As such, it is believed that the difference in feed intake or growth according to feeding frequency leads to different results depending on the size and length of the intestine, the diet of the fish, the type or physical properties of the feed, and the breeding environment (NRC, 1993; Lee et al., 2000b). PER was significantly lower in the experimental groups that received three or four meals per day than in those that received one or two meals per day. Previous studies reported that protein efficiency declined as feed supply increased due to an excessive intake of energy, and PER of olive flounder and black rockfish declined when feeding frequencies were over optimum levels (Kim et al., 2009; Lee et al., 2013), which were consistent with the results of the present study.

The increase in lipid content with increasing feeding frequency was similar to that observed in red-spotted grouper (*Epinephelus akaara*)(Kayano et al., 1993), Korean rockfish (Lee et al., 2000b) and rock bream Oh and Maran 2015). This increase occurs because the energy in the extra food consumed by the fish from frequent feeding is not used for growth and is stored as body fat (Mizanur and Bai 2014; Kim et al., 2020). Xie et al. (2011) reported that sufficient feeding due to increased feeding frequency can accumulate a higher fat content in the body because it consumes less of the energy required to avoid competition and cannibalism for feeding.

Based on the growth performance, feed intake and body composition, the optimum feeding frequency for growth of juvenile mud loach with a body weight of 1 to 5 g was two meals a day.

<Table 1> Ingredient and proximate composition of experimental diets for mud loach *Misgurnus mizolepis*

Ingredients (%)	Diets
Commercial diet[1]	
Chemical analysis (% of dry matter basis)	
Moisture	24.8
Crude protein	57.1
Crude lipid	11.3
Ash	8.4

[1]Fish Commercial diet for mud loach produced from Chunhajeil incorporation (Daejeon, Korea).

<Table 2> Growth performance of mud loach *Misgurnus mizolepis* fed experiment diets for 10 weeks[1]

Feeding frequency /day	Initial mean weight (g)	Final mean weight (g)	Survival (%)	Weight gain (%)[2]	Specific growth rate (%/day)[3]
One meal	1.0±0.01[ns]	2.5±0.05[a]	100±0.0[ns]	147.2±5.0[a]	1.29±0.03[a]
Two meals	1.0±0.01	4.5±0.15[b]	100±0.0	350.0±16.9[b]	2.15±0.06[b]
Three meals	1.0±0.01	5.0±0.45[b]	100±0.0	397.9±41.3[b]	2.29±0.12[b]
Four meals	1.0±0.01	5.4±0.01[b]	100±0.0	440.9±5.35[b]	2.42±0.02[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 mud loach *Misgurnus mizolepis* fed experiment diets for 10 weeks[1]

Feeding frequency /day	DFI(%)[2]	FE(%)[3]	DPI(%)[4]	PER(%)[5]
One meal	1.83±0.11[a]	26.1±2.45[a]	1.00±0.06[a]	0.48±0.05[a]
Two meals	2.09±0.12[a]	78.5±6.80[b]	1.11±0.06[a]	1.43±0.13[b]
Three meals	2.70±0.30[b]	83.2±0.50[b]	1.45±0.09[b]	1.51±0.01[b]
Four meals	2.73±0.20[b]	69.6±3.95[b]	1.50±0.11[b]	1.08±0.70[b]

[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 consumed.

<Table 4> Condition factor (CF), coefficient variation of body length (CVBL), and body weight (CVBW) of mud loach *Misgurnus mizolepis* fed experiment diets for 10 weeks[1]

Feeding frequency /day	CF(%)[2]	CVBL(%)[3]	CVBW(%)[4]
One meal	0.49±0.30[ns]	101±0.1[ns]	33.9±4.4[ns]
Two meals	0.56±0.11	9.7±0.6	29.5±2.9
Three meals	0.53±0.12	9.6±0.4	34.1±3.1
Four meals	0.54±0.20	9.5±0.4	30.9±3.0

[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 mud loach *Misgurnus mizolepis* fed experiment diets for 10 weeks[1]

	Diets			
	One meal	Two meals	Three meals	Four meals
Proximate composition (% wet weight)				
Moisture	72.2±1.4[ns]	72.6±0.2	72.1±1.9	71.7±0.5
Crude protein	18.0±0.9[ns]	17.6±0.1	17.2±1.3	17.7±0.2
Crude lipid	3.95±0.05[a]	4.95±0.05[ab]	5.55±0.75[ab]	6.30±0.10[b]
Ash	4.65±0.15[ns]	4.60±0.20	4.80±0.10	4.55±0.05

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

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