Feeding Regimes Of The Small Minnow In Recirculating Water System: The Pilot Project to Culture Thai Indigenous Minnow *Rasbora borapetensis* for Using in The Biomedical Research

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ABSTRACT

The National Committee for Research Animal,Thailand had launched the pilot project to study the possibility to breed and culture the Thai indigeneous minnows in recirculation water system in order to reduce the import of zebrafish from abroad to study the medical research. The feeding trial of larvae with different stages, juvenile and broodstock had been investigated including the measurement of mouth size of larvae with the age of 3-15 days. The feeding trail had been conducted in the circulating water system with acrylic tank set up on the shelf, water had been filtered using glass wool, plastic bio-ball and disinfected by ultraviolet. The mouth size of the first feeding period was around 56 micron, then on the age of 1 week the mouth size increased around six times to be around 355 micron, then at the age of 2 weeks the mouth size increased about 28 times of the first feeding size to be about 1,567 micron. The survival rate of the fry with the age of 3-10 days quite low as this was the first breeding success, the survival rate of fry fed with cholera, brachionus and powder feed were 5%, 7% and 10% respectively which was not significantly different (P>0.05). While the survival rate of the fry with the age of 11-30 days was higher than the younger one, the highest survival rate obtained from fry fed powder feed with 37% while the fry fed Moina and Artemia was not significantly different (P>0.05) in survival rate, it was 20% and 22% respectively. Both survival rate and growth performance of juvenile fed with different feeds were not significantly different among treatments (P>0.05). There were no significantly different (P>0.05) in growth rate,GSI and number of egg. Fish fed pellet feed coated with squid oil had the higher GIS than the others, while the lowest GIS was obtained from fish fed Moina.

Keywords: recirculation water system, feeding period, survival rate

INTRODUCTION

For conducting the biomedical research, the scientist using some kind of vertebrates as animal model. Zebrafish is more common for using in the biomedical research including in Thailand, it is indigenous to South Asia. Every year Thailand have to import for aquarium business and biomedical research. Therefore the National Committee for Research Animal,Thailand had launched the pilot project to study the possibility to breed and culture the Thai indeginous minnows in recirculation water system. The characteristics of zebrafish which are favors for using in biomedical research i.e. small size, fast development and visional transparency during early development (Lawrence, 2007). Following those characteristics, Thai small indigenous minnows *Rasbora borapetensis* or black line rasbora had been selected for this study. It has been commonly found in the small stream, man-made pond and swamp with sandy soil. Most of the black line rasbora has been fishing for aquarium business as it has bright red caudal fin. The analysis of stomach content showed omnivorous characteristic of feeding habit but prefer animal. (Payooha *et a*l, 2011). While there are several reports of breeding and culture of other small minnows but the data of these species are lacking. To study on feeding management of these two species in recirculation system and simulated the similar condition as in the biomedical studies is the challenge. Thus the purpose of this study was to investigate the feeding regimes of this indigenous minnows in the recirculating water system.

METHODOLOGY

Mouth size measurement

Newly hatched larvae is critical period of fish, poor feeding management leading to low survival rate. Food preference is one of the most important factor for survival so the measurement of mouth size is necessary. The mouth size was measured following Shirota (1970), gap of the jaw was measured while mouth open to an angle of 90°.

Feeding Trials

The feeding trials for fry, juvenile and broodstock had been conducted in the small acrylic tank with recirculating water system. The tank size for fry trial was 14 cm×18 cm×20 cm. contained 5 -ball and disinfected by ultraviolet. The feeding s and 11-30 days. There were 3 types of feed

using during the first phase of feeding trial i.e. *Chlorella sp*., *Brachionus sp*. and commercial powder feed with 40% protein content. The fry with the age of 3 days post hatching were removed from the breeding tanks located in the same room to the experimental tanks weighted and randomly stocked 100 individual per tank in triplicate.

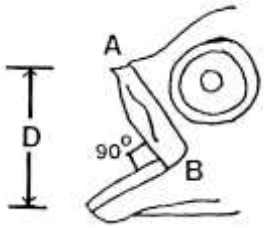

Figure 1. Mouth size (D) = √2 AB

The larvae were fed 5 times daily, 10 ml of the live food was provided each time while the powder feed had been used 10%BW/day. The density of *Chlorella sp***.** and *Brachionus sp*.were $2x10^7$ cell/ml and 50 individual/ml respectively. The second phase (11-30 days) of fry feeding trial using the following feed i.e. larvae of *Artemia sp.*, larvae of *Moina sp.* and commercial powder feed with 40% protein. Fish were fed 4 times daily, the larvae of *Artemia sp*. and *Moina sp*. were provided 10 g. each time while powder feed had been used 10%BW/day. Fish with age of 3 days were weighted and randomly stocked 50 individual per tank. The juvenile feeding trial was set up using the bigger tank with size of 24×24×30 cm contained 20 liters of water. For the juvenile feeding trial, fish were collected from the swamp in Ubon Ratchathani province then acclimatized in the tank for 1 week before stocking for the feeding experiment. Fish were weighted and randomly stocked 30 individual per tank. Feed using in the juvenile trial were the same as in the second phase of fry trial. Fish were fed 3 times daily for 12 weeks, the adult of *Artemia sp*. and *Moina sp*. were provided 30 g. each time while powder feed had been used 3%BW/day. For the feeding trial of broodstock, the wild fish were collected from the swamp in Ubon Ratchathani province then acclimatized and feeding with pellet feed with 40% protein for 4 weeks before conducting the feeding experiment for 12 weeks. Fish were weighted and randomly stocked 10 individual per tank. There were 5 feeding treatments i.e. pellet feed coated with squid oil, pellet feed coated with squid oil and soybean oil, pellet feed coated with soybean oil, *Moina sp.* and *Artemia sp.* Feeding ration was 3%BW/day for the group fed with pellet feed while the live foods were provided 60 g. 2 times daily. The important water quality parameter were monitored the whole period of experiments.

Data Analysis .

Analysis of variance (ANOVA) was conducted at 0.05 significant level to compare specific growth rate (SGR), %weight gain (%WG), %survival rate (%SR) and gonadosomatic index (GSI) **.** The statistical analysis were carried out using SPSS 10.0.1

RESULT

The mouth size of larvae with different age was showed in Table1. From closely observation during the post hatching period, the most critical period during nursing is 1-15 days of age so the mouth size of larvae at those age had been measured for the efficient food selection. As the yolk sac absent on the day 3,the first feeding begin. The mouth size of the first feeding period was around 56 micron, then on the age of 1 week the mouth size increased around six times to be around 355 micron, then at the age of 2 weeks the mouth size increased about 28 times of the first feeding size to be about 1,567 micron.

Table 1. The mouth size of larvae with different ages.

Species	Age (Day)				
	3	4-6	7-9	10-12	13-15
Rasbora borapetensis	0.056±0.009	0.100±0.017	0.355±0.051	1.000±0.038	1.567±0.075

The survival rate of the fry with the age of 3-10 days quite low as this was the first breeding success, the survival rate of fry fed with Chlollera, Brachionus and powder feed were 5%, 7% and 10% respectively which was not significantly different (P>0.05). While the survival rate of the fry with the age of 11-30 days was higher than the younger one, the highest survival rate obtained from fry fed powder feed with 37% while the fry fed Moina and Artemia was not significantly different (P>0.05) in survival rate, it was 20% and 22% respectively.

The growth performance of juvenile fish fed with different feeds for 12 weeks showed in Table 2. Both survival rate and growth performance of juvenile fed with different feeds were not significantly different among treatments (P>0.05). The survival rate of juvenile was higher than the fry for all treatments. The growth curve of juvenile fed different feeds showed in Fig. 2, at the end of fourth weeks fish fed with pellet feed show better growth rate than the others. During the period of fourth week to eighth week, fish fed Moina showed the sharp increase of growth rate. From the period of eighth week to the end of feeding trial, fish fed Artemia had the sharp increase of growth rate.

Table 2. The growth performance of juvenile fish fed with different feeds.

Feed/Growth	Final Weight (g)	ADG (g)	SGR (%/day)	SR (%)
Powder Feed	0.16 ± 0.150	0.0010±0.006	4.9604±1.14	38.88±1.92
Moina sp.	0.15 ± 0.122	0.0010±0.005	4.8832±0.97	41.11±6.93
Artemia sp.	0.14± 0.034	0.0005±0.003	5.8494±0.89	34.44±5.09

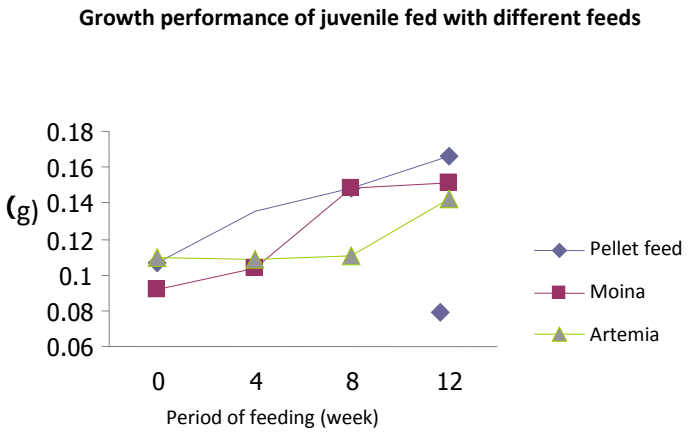

Figure 2. Growth performance of juvenile fed different feeds for 12 weeks

The result of feeding trial of broodstock showed in Table 3. There were no significantly different (P>0.05) in growth rate,GSI and number of egg. Fish fed pellet feed coated with squid oil had the higher GIS than the others, while the lowest GIS was obtained from fish fed Moina.

Table 3. Growth and reproductive performance of broodstock fed different feeds

Parameters	Type of Feed				
	Commercial Feed coated with squid oil 100%	Commercial Feed coated with squid oil +soybean oil (1:1)	Commercial Feed coated with soybean oil 100%	*Moina sp.*	*Artemia sp.*
SGR(%/Day)	3.16±1.36	2.14±0.06	3.07±0.43	3.09±0.70	3.30±0.69
GSI (%)	10.76±3.05	9.70±3.15	6.70±2.16	4.91±1.70	7.13±1.30
			386.67±82.86	387.00±97.61	420.33±108.48

DISCUSSION

From the result of mouth size of larvae during first feeding which was around 56 micron so the size of food which is smaller than the larvae mouth is Chrollela and powder feed which the size of Chrollera is about 2.3-3.5 micron, rotifer 79-117 micron, Moina 250-350 micron and Artemia 400-500 micron (Wongrat,2000). But the survival rate of larvae fed Chrollera was the lowest, this could be because of from stomach content analysis of wild fish found most of food items were animal i.e. Moina and insect so the larvae which had omninivorous characteristic should prefer animal than plant and the stomach shape showed more carnivorous characteristics(Payooha *et al,* 2012). But to better understanding on feeding habit of black line rasbora, the enzyme activity analysis should be investigated at different age to proper feed preparation which lead to the high survival rate. The food for larvae with the age of 7-9 days can be Chrollera, Moina and rotifer which have smaller size than fish mouth. At the age of 10-15 days other than those feed, Artemia can be used to feed the larvae as the size smaller than fish mouth.

The commercial powder feed with 40% protein should be attractive to the fish larvae as the strong smell of fish meal attracted the larvae and during first period of feeding the larvae was to weak to swim to catch the food but the powder feed floating all over area so could make the larvae ingest easily. For the feeding trial of juvenile although there were no significantly different (P>0.05) among treatments due to high standard deviation but the group of fish fed pellet feed had the trend for better growth rate as the juvenile using in this trial were the wild population which may be stressed in the new environment lead to slowly swimming to catch the live food while pellet feed floating all over area, so fish can catch easily and the strong smell of fish meal also should be another factor to attract the fish. When compare to the study of zebra fish which used the fish from aquaculture found that the fish fed live food either Artemia or Paramecium had the higher growth rate (Goolish *et al*, 1999 and Carvalho *et a*l., 2006). The result of both reports showed the same trend as the review of Lawrence (2007). As the zebra fish had been bred in aquarium for a long period of times so the fish quite acclimatize to the aquarium environment so could adapt to catch the live food which is high nutritional and suitable for the fish (Wanatabe *et al*., 1983). Fish also had higher digestibility of live food (Cahu and Zambonino Infante, 2001).

For the feeding trial of broodstock, although there were no significantly different among treatments (P>0.05) but the fish fed pellet feed coated with squid oil and fish fed Artemia had the trend of better reproductive performance. The squid oil and Artemia contain high highly unsaturated fatty acids (HUFA) especially docosahexaaenoic acid (DHA) and eicosapentaenoic acid (EPA) which both fatty acids has the important role in reproductive system of invertebrate (Muskiet *et al.,* 2006) and the study of Furuita *et al*., 2002 and Rennie *et al*., 2005 also showed the lucid result of the affect of HUFA on the reproductive system of fish.

From the result of this study, to culture the Thai small minnows in recirculating water system for using in medical research has the high potentiality but need more study on enzyme activity of digestive organ for better understanding of feeding biology. And the breeding section also has to investigate more to get more number of fish for using as broodstock within the circulating system no need to collect from the wild.

ACKNOWLEDGEMENT

The authors would like to thank the National Committee for Research Animal, NRCT Thailand for financial support and also extend thank to the Faculty of Agriculture, Ubon Ratchathani University for provision the laboratory for this study.

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