J Krishi Vigyan 2020 (Special Issue) : 90-95 DOI : 10.5958/2349-4433.2020.00087.2

Evaluation of Microparticulate Diets for Larval Rearing of Endangered Fish, Golden Mahseer (*Tor putitora)*

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ABSTRACT

In the present study, performance of microparticulate diets supplemented with exogenous dietary digestive enzyme mix was compared with live feed *Artemia* nauplii for the feeding of golden mahseer larvae after 15d of yolk sac absorption. One thousand five hundred golden mahseer larvae were randomly distributed in five treatment groups (100 larvae per tank) following a completely randomized design. Larvae were manually fed either *Artemia* nauplii or macerated goat liver or microparticulate diets supplemented with exogenous dietary digestive enzyme mix at different levels (0, 2.0 and 4.0 g/kg diet). After 60 d of feeding, no significant ($P \geq 0.05$) difference was noticed in the survival of golden mahseer larvae fed with different diets. However, there was significant ($P \leq 0.05$) effect of different dietary treatments on the weight gain and specific growth rate. The highest weight gain and specific growth rate was observed in the *Artemia* nauplii fed group followed by microparticulate diet containing digestive enzyme mix of 2.0 g/kg diet. The weight gain of larvae fed the microparticulate diet supplemented with digestive enzyme mix of 2.0 g/kg diet was almost 80.4 per cent of that achieved for larvae fed *Artemia* nauplii. The lowest weight gain per cent and specific growth rate was evidenced in the group fed with macerated goat liver. Based on the results of the present study, the microparticulate diet supplemented with exogenous dietary digestive enzyme mix (2.0 g/kg diet) can be an alternative for the feeding of golden mahseer larvae after 15 days of yolk sac absorption.

Key Words: Golden mahseer, Growth, Live feed, Microparticulate diet, Survival.

INTRODUCTION

Golden mahseer (*Tor putitora*) is an important cyprinid fish, once contributed a significant proportion of the natural stock of fish in India. In recent years, due to anthropogenic pressure, pollution, environmental degradation and indiscriminate fishing, the population of golden mahseer in the natural water bodies has declined to an alarming level from various parts of Asia (Akhtar *et al*, 2014). It is now identified as a critically endangered species in the IUCN Red List of Threatened Species (IUCN, 2015). Therefore, it is need of the hour to give special attention to conserve the population of this species through artificial breeding, seed rearing and finally ranching into its natural habitats. The major bottleneck in its successful seed rearing is unavailability of suitable larval diets. Previous study on activities of digestive enzymes indicated that mahseer larvae are able to digest protein, lipid and carbohydrate at an early stage (Akhtar *et al*, 2013). The results revealed that micro-particulate diets could be formulated for rearing of golden mahseer larvae from 15 days after hatching taking into account the digestive capacity (Akhtar *et al*, 2013; Sharma *et al*, 2016).

Live food organisms have been considered as the most suitable feed for successful rearing of fish and crustacean in their early larval stages, but there are many problems inherent to it (Kovalenko *et al*, 2002). Some of the issues associated with live food include variable nutrient composition and availability, introduction of potential pathogens into the culture system, the high cost of production and thereby substantial increases in the production cost involved in hatchery operation (Kovalenko *et al*,

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2002). Several attempts have been made to develop formulated diets that effectively replace live food but formulated diets are used as supplements because when used exclusively, growth and survival are often compromised (Kovalenko *et al*, 2002). Reasons for difficulties in successful rearing of the early stages of fish larvae on artificial diets are not specifically known but several factors are thought to play important role. The main reason is that the digestive systems of larvae are usually not fully developed and may not possess sufficient digestive enzyme activity necessary for effective digestion of artificial diets (Kolkovski, 2001). Cahu and Zambonino-Infante (2001) suggested that it will be necessary to formulate diets that are specifically designed to complement the digestive physiology of fish larvae. However, there is very little study on the use of exogenous enzymes in the larval diet of golden mahseer. Therefore, in the present study, an attempt has been made to develop a microparticulate diet supplemented with enzymes to substitute live food or goat liver for larval rearing of golden mahseer.

MATERIALS AND METHODS

Golden mahseer larvae

Golden mahseer larvae of 12d post yolk sac absorption were procured from ICAR-DCFR mahseer hatchery, Bhimtal, Nainital, Uttarakhand and brought to wet lab of ICAR-DCFR. The larvae were kept in a rectangular 1000 L FRP tank with sufficient aeration. After three days, the larvae (average weight 18.0 ± 2.7 mg) were distributed to experimental units of 100 L capacity. One thousand five hundred golden mahseer larvae were randomly distributed in five treatment groups in triplicates (100 larvae/tank of 100 L capacity) following a completely randomized design. The five treatment groups were: T-artemia – fed with *Artemia* nauplii; T-liver – fed with macerated goat liver; T-mpd0 – fed with microparticulate diet without exogenous digestive enzyme mix supplementation; T-mpd1 – fed with microparticulate diet with exogenous digestive enzyme mix level 1 supplementation (2.0 g/kg diet) and T-mpd2 – fed with microparticulate diet with exogenous digestive enzyme mix level-2 supplementation (4.0 g/kg diet). The physico-chemical parameters of water were within the optimum range (dissolved oxygen: 5.6–6.4 mg/L; pH: 7.4–8.5; ammonium nitrogen: 0.043–0.069 mg/L; nitrate: 2.9 – 6.5 mg/L; free $CO_{2.}$ 6.0 – 10.0 mg/L; total alkalinity: 120 – 128 mg/L; total hardness: 148 – 152 mg/L; phosphate: 0.23 – 1.20mg/l; chloride: 13 – 33 mg/L: sulphate: 23 – 63 mg/L; potassium: 5.5 – 7.6 mg/L; silicate: 26 – 42 mg/L and iron: 0.01 – 0.23 mg/L) throughout the experimental period of 60 d. A water flow of 1.0 L/min was maintained and continuous aeration was provided to ensure the optimum level of dissolved oxygen. All the groups were fed their respective diets *ad libitum* thrice a day. The uneaten feed and faecal matters were removed by siphoning and about 50 per cent water of the tanks was exchanged daily with borewell water to maintain the optimum water quality.

Diet preparation and proximate analysis

The ingredient and proximate composition of the formulated microparticulate diets tested (Table 1). Fish meal, groundnut meal, casein, gelatin, rice starch, soy lecithin, egg albumin powder, wheat flour and sodium alginate were added together and mixed thoroughly in a mixer. After mixing, fish oil and attractants (betaine hydrochloride and choline chloride already dissolved in water) were added to it and mixed well and made it into semi-solid dough by adding sufficient amount of water. The resulting dough was autoclaved for 20 min followed by cooling. Subsequently vitamin-mineral mix, vitamin C and exogenous digestive enzyme mix were added and mixed thoroughly. The resultant dough was spread as a uniform thin layer on a tin tray and dried in a hot air oven over night at 60 ^{o}C. The dried feeds obtained were pulverized and sieved to get uniform sized particles of 300-350 micron equivalent to the size of *Artemia* nauplii. The proximate composition of the experimental diets was determined following the standard methods of AOAC (1995).

Table 1. Percentage inclusion of ingredients and proximate analysis of diets (% dry weight basis).

Ingredient	Microparticulate diets		
	T-mpd0	T-mpd1	T-mpd2
Fish meal[a]	15.0	15.0	15.0
Groundnut meal[a]	15.0	15.0	15.0
Casein[b]	10.0	10.0	10.0
Gelatin[b]	03.0	03.0	03.0
Rice powder[a]	08.0	08.0	08.0
Soy lecithin[b]	03.0	03.0	03.0
Egg albumin powder[b]	16.0	16.0	16.0
Wheat flour[a]	12.0	12.0	12.0
Fish oil[a]	08.0	08.0	08.0
Vitamin - mineral mix[c] (1:1)	05.0	05.0	05.0
Vitamin C[b]	0.1	0.1	0.1
Betaine hydrochloride[b]	0.25	0.25	0.25
Choline chloride[b]	0.25	0.25	0.25
Sodium alginate[b]	04.0	04.0	04.0
Digestive enzyme mix* (g/kg)	-	2.0	4.0
Proximate analysis (Mean ± SE, n = 3)			
Moisture	8.4±016	8.47±0.92	8.17±0.47
Crude protein	40.53±1.43	40.76±0.71	41.73±0.72
Ether extract	8.40±0.27	8.50±0.57	8.58±0.28
Ash	12.59±0.79	11.80±0.27	11.89±0.21
Dry matter	91.60±0.15	91.53±0.93	91.82±0.48

[a]Procured from local market, [b]Himedia Laboratories Ltd, Mumbai, India, [c]Prepared manually and all components from Himedia Ltd, India.

*The digestive enzyme mix was made manually containing trypsin and pepsin powder (1:1). The trypsin powder contained chymotrypsin – 70 – 140 unit/mg and trypsin – 1000 – 1500 units/mg. The pepsin powder contained 3000 units pepsin/mg with an activity of 1 anson unit/g. The required digestive enzyme mix was mixed thoroughly with vitamin-mineral mixture and then added in the diet. The trypsin and pepsin powders were procured from HiMedia Laboratories Ltd. Mumbai, India.

Freshly collected goat liver from the local market was first washed thoroughly and then boiled. The outer membrane and hepatic veins were removed from the cooked goat liver followed by its maceration with the help of indigenous stone slate and pestle. This macerated goat liver was spread on a stone and put in the rearing tank for feeding the larvae.

Approximately 10 g *Artemia* cysts (M/s S. K. Trading, Bangkok, Thailand) were placed in water at room temperature for approximately one hour with constant aeration to keep the cysts well suspended. After one hour of hydration, the water and hydrated cysts were drained through a sieve and the cysts were then placed into a beaker. Chilled sodium hydroxide solution was added into the beaker

containing hydrated cyst and adequate aeration was provided to keep cysts suspended. When the cysts were adequately decapsulated, sodium thiosulfate was added to the decapsulated cysts to neutralize the chlorine. Aeration was stopped to settle the decapsulated cysts at the bottom and was siphoned through air tubing and was then washed thoroughly with water. The decapsulated cysts were stored in refrigerator for two weeks for subsequent hatching. For hatching, approximately 1 g of decapsulated cysts was placed into a 5L beaker containing water of 25 ppt salinity at 26 °C. Illumination was provided by lighting a 100 W bulb. After 16-20 hr, the hatched nauplii were collected by draining the entire water into a meshed bag and rinsed with running water for 2 to 3 min and was fed to the fishes of the respective experimental group.

Growth and survival study

Growth rate and survival of golden mahseer larvae were measured in terms of weight gain (%) and percentage specific growth rate (% SGR) and percentage survival using the following equations.

Weight gain (%) = [(Final weight – Initial weight) / Initial weight] x 100

SGR (%) = [(ln final weight – ln initial weight) / Number of experimental days] x 100

Survival % = [Total no. of larvae at the end of the trial/ Total no. of larvae stocked] x 100

Evaluation of water stability and dry matter loss of microparticulate diet

After the experimental trial and considering the performance of the microparticulate diets, the water stability and dry matter loss of microparticulate diet T-mpd1 (the diet supplemented with exogenous digestive enzyme mix @2.0 g/kg) was determined following the method of wet durability test reported by Ighwela *et al* (2013) with considerable modifications to ensemble the present conditions. Weighed samples (1-2 g) of microparticulate diet were dropped into previously weighed glass beakers containing 800 ml of distilled water in triplicates for each time point. The immersion times examined were 30 min, 60 min and 120 min. After immersion, the undissolved solids and water were filtered and the filtrates were put back in the respective beaker. The beakers with the undissolved solids were dried in the oven (105 °C for 30 min), followed by further drying at 65 °C over night to a constant weight and then cooled in desiccators. The mean differences in weights of beakers containing the feed before immersion and after drying were used to calculate the percentage dry matter loss, which is a measure of the water stability of the feed for the corresponding time intervals.

The % loss of dry matter was determined by using following formula:

% dry matter loss =

100 - [(Weight of beaker + sample after drying) – Weight of empty beaker] / weight of sample taken X 100

Statistical Analysis

Mean values of all parameters were subjected to one-way ANOVA to study the treatment effect and Duncan's Multiple Range Tests were used to determine the significant differences between any two means. Comparisons were made at 5% probability level. All the data were analyzed using statistical package SPSS (Version 19).

RESULTS AND DISCUSSION

Growth and survival

There was no significant ($P \geq 0.05$) effect of different dietary treatments on survival of golden mahseer larvae (Fig. 1). However, the highest survival was seen in T-artemia followed by T-mpd1 and T-mpd2. On the other hand, there was significant ($P \leq 0.05$) effect of different dietary treatments on the weight gain % (Fig. 1) and specific growth rate (Fig. 2) of golden mahseer larvae at the end of 60 days of feeding. The highest weight gain and specific growth rate was observed in the T-artemia group fed with *Artemia* nauplii as reference diet followed by

T-mpd1 group which was fed with microparticulate diet with exogenous digestive enzyme level 1 supplementation (2.0 g/kg diet). Weight gain in T-mpd1 group was almost 80.4 % of that achieved for larvae fed *Artemia* nauplii as reference diet. The lowest weight gain and specific growth rate was evidenced in the group T-liver fed with macerated goat liver. The weight gain and SGR % in T-mpd0 and T-mpd2 were similar but significantly lower than T-mpd1.

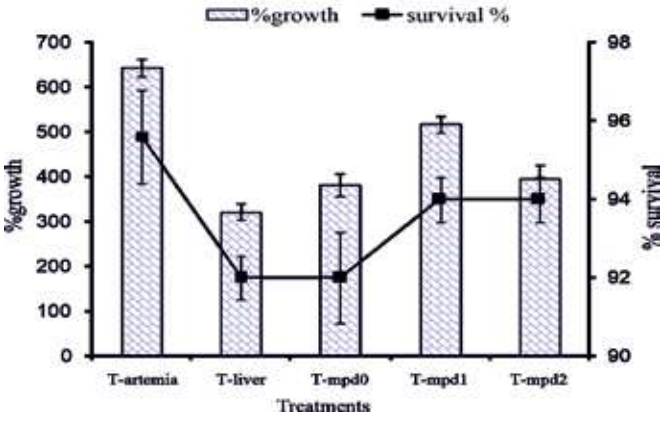

Fig 1. Growth and survival of golden mahseer larvae fed with different diets during 60 days of rearing. (Values in the same series with different superscript (a, b, c) differ significantly (P≤0.05; Data expressed as Mean ± SE, n = 3).

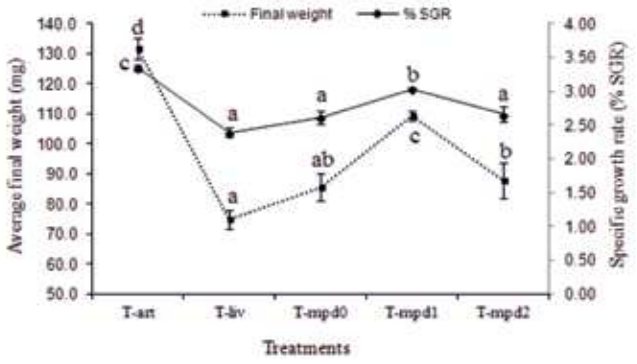

Fig 2. Percentage specific growth rate (% SGR) and average final weight of golden mahseer larvae fed with different diets for 60 days. (Values in the same series with different superscript (a, b, c) differ significantly (P≤0.05; *Data expressed as Mean ± SE, n = 3)*.

It was evident that the endogenous enzyme activity in golden mahseer larvae was not sufficient for the digestion of microdiets as indicated by the low growth performance of T-mpd0 group. However, supplementation of dietary digestive enzyme mix in the micro diet positively affected the larval growth. Better growth performance of golden mahseer larvae fed enzyme mix supplemented micro diet in the present study supports the findings of several authors in different species (López-Alvarado, 2015; Patil and Singh, 2014). This may be due to the increase in digestive capacity of larvae because of the ready availability of digestive enzymes along with the feed. Additionally, supplementation of exogenous enzymes may lead to the activation of endogenous enzymes or zymogens in the digestive system and might have resulted in increased digestive capacity and nutrient utilization in larvae. Improved growth and feed utilization was reported in the larvae of *Cyprinus carpio* by exogenous supplementation of trypsin in its larval diets (Dabrowska *et al*, 1979). Further, this study revealed that low level incorporation of dietary digestive enzyme mix in the larval diet of golden mahseer resulted in better growth suggesting development of cost effective larval diet.

Evaluation of dry matter loss

The data on per cent dry matter losses at different durations of immersion is presented in Fig. 3. The results indicated that there was significant (P≤0.05) effect of immersion time on per cent dry matter loss of tested microparticulate diet. The minimum loss was observed at 30 min of immersion and there was a linear increase ($y = 2.96x + 12.803$, $R^2 = 0.9633$) in per cent dry matter loss with the duration of immersion. Most of the microparticulate diet tested in fish has been manifested with high leaching rate of nutrients (Kolkovski, 2008). Microdiets that are hard and leach resistant will be a challenge to the larvae digestive systems, while a diet that will be digested easily in the gut will also disintegrate relatively fast in the water (Yufera *et al*, 2008). Therefore, it is essential to have a balance between nutrient leaching and digestibility of particles. The microparticulate diet used in the present study showed acceptable water stability and digestibility.

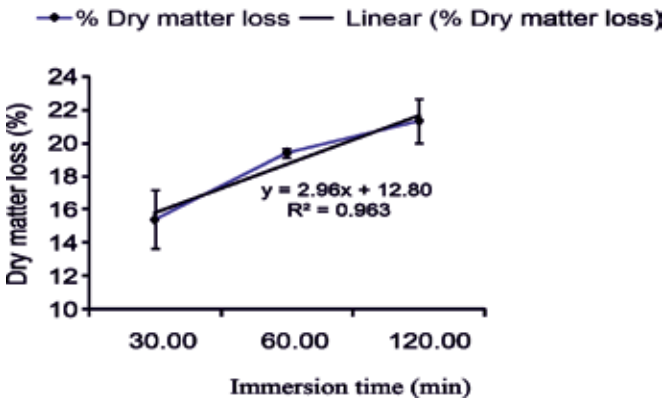

Fig 3. Percentage dry matter loss of tested microparticulate diet at different duration of immersion (*Data expressed as Mean ± SE, n = 3)*.

CONCLUSION

The overall results of the present study demonstrated that the growth and survival of golden mahseer larvae is highest when fed with live feed *Artemia* nauplii. However, the microparticulate diet supplemented with exogenous dietary digestive enzyme mix (2.0g/kg diet) gives nearly comparable growth with similar survival. Hence, the microparticulate diet supplemented with exogenous dietary digestive enzyme mix @ 2.0 g/kg diet is an alternative for the feeding of golden mahseer larvae after 15 d of yolk sac absorption. These findings will decrease our dependency on live feed and will help in development of cost-effective, reliable and easy hatchery production protocols of golden mahseer. However, future research is still required to develop a suitable weaning diet for complete replacement of live feed/goat liver.

ACKNOWLEDGMENTS

We acknowledge the Director, ICAR-DCFR, Bhimtal, for providing facilities for the study. Authors are also thankful to Mr. Santosh Kumar for the technical assistance provided. This study was funded under the ICAR Outreach Activity Consortium#1: Fish Feed.

REFERENCES

Akhtar M S, Sarma D and Pandey N N (2013) Activities of digestive enzymes during ontogenic development of golden mahseer *(Tor putitora)* larvae. *Indian J Anim Sci* **83**: 560–563.

Akhtar M S, Pal A K, Sahu N P, Ciji A and Mahanta P C (2014) Higher acclimation temperature modulates the composition of muscle fatty acid of *Tor putitora* juveniles. *Weather and Climate Extremes* **4**: 19-21.

AOAC (1995) Official Methods of Analysis of the Association Official Analytical Chemists. In: Cunniff PA (ed) vol. 1, 16th edn. AOAC International, Arlington, Virginia, USA, 31–65.

Cahu C and Zambonino-Infante J (2001) Substitution of live food by formulated diets in marine fish larvae. *Aquaculture* **200**: 161–180.

Dabrowska H, Grundniewski C and Dabrowski K (1979) Artificial diets for common carps: effect of the addition enzyme extracts. *Prog Fish Cult* **41**: 196-200.

IUCN (2015) IUCN red list of threatened species, Version 2015.2 www.iucnredlist.org. Accessed on August 26, 2015.

Kolkovski S (2001) Digestive enzymes in fish larvae and juveniles-implications and applications to formulated diets. *Aquaculture* **200**: 181-201.

Kolkovski S (2008) Advances in marine fish larvae diets. In: Cruz-Suárez E, Ricque D, Tapia M, Nieto MG, Villarreal LDA, Lazo JP, Viana MT (eds) Avances en Nutrición Acuícola IX, Universidad Autónoma de Nuevo León, Mexico, Nuevo León, Pp. 20-45.

Kovalenko E E, D'Abramo L R, Ohs C L and Buddington R K (2002) A successful microbound diet for the larval culture of freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture* **210**: 385–395.

López-Alvarado J (2015) Effects of Dietary Enzymes on the Larval Performance of the red seabream (*Pagrus major*). *Int J Sci Res* **4**: 24-27.

Patil D W and Singh H (2014) Effect of papain supplemented diet on growth and survival of post -larvae of *Macrobrachium rosenbergii*. *Int J Fish Aquat Stud* **1**: 176-179.

Sharma P, Akhtar M S, Singh A K, Das P and Sarma D (2016) Histo-morphological changes in digestive tract of golden mahseer (*Tor putitora*) during different developmental stages. *Fish Physiol Biochem* **42**(6): 1681-1698.

Yufera M, Fernadez-Diaz C and Pascual E (2005) Food microparticles for larval fish prepared by internal gelation. *Aquaculture* **245**: 253-262.

Received on 25/06/2020 *Accepted on 15/08/2020*