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Evaluation of single and multiple-ingredient Feeds for 7-d Post-hatch *Osteobrama belangeri* Larvae in Clearwater System

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ABSTRACT

Feeding trial was conducted to evaluate the growth performance and survival of 7-d post-hatch (dph) *Osteobrama belangeri* larvae on four different allochthonous feeds namely; a simple mixture of mustard oil cake and rice bran (1:1; D1), a multiple ingredient-based compound feed (D2) of rice bran, mustard oil cake, soybean meal, fishmeal and, vitamins and minerals (3.5:2:2:2:0.5, D2); crushed fresh *Wolffia globosa* (D3) and dry meal of *W. globosa* (D4). The trial was conducted for 21 days in clearwater system inside a wet laboratory. The 7-d old *O. belangeri* larvae (mean weight 2.0±0.04 mg; mean length 5.50±0.50 mm) were stocked @120 numbers in glass aquariums (60 cm×30 cm×30 cm) filled with 40 L conditioned groundwater and provided with continuous aeration. Larvae were fed three times a day (8.00 AM, 12.00 PM and 4.00 PM) at combined feeding rate @100%-200% of their biomass (dry matter basis) by offering 1/3 of daily ration on each occasion. The final mean weight, final mean length and specific growth rate of *O. belangeri* were significantly highest (p<0.001) for D2 (73.70±1.53 mg and 12.83±0.44 mm) followed by those of D1 (53.37±3.07 mg and 11.67±0.17 mm), D4 (46.03±2.11 mg and 10.17±0.33 mm) and D3 (27.87±0.58 mg and 9.83±0.44 mm), in order. Survival of *O. belangeri* varied between 30-62% but no significant differences (P>0.05) were established between treatments. The results of the present study indicated that 7-dph *O. belangeri* larvae could utilise dry meal of Wolffia as a single-ingredient feed in clearwater system.

Key Words: Growth performance, Larval feed, *Osteobrama belangeri,* Survival, Wolffia.

INTRODUCTION

In recent years, *Osteobrama belangeri*, commonly known as pengba, has emerged as an important candidate species for freshwater aquaculture species diversification in India due to perceived superior flesh quality, taste likability, high price, high demand, high degree of compatibility with other fishes, especially Indian major carps and low in food-chain feeding behaviour (Rathod *et al*, 2014; Kumar *et al,* 2017). Considering near-threatened IUCN status of its natural population in India (Vishwanath, 2010) *vis-à-vis* extreme preference by local populace of the state, *O. belangeri* has been designated as the "State Fish" of Manipur. Establishment of aquaculture of this species, especially in north-eastern region is valuable for not only food and nutritional security but also livelihood security of the region.

An assured supply of sufficient quantity of *O. belangeri* seed is a pre-requisite to realisation of the potential of this species. Notably, development and establishment of efficient larval rearing is one of the most critical steps in seed production of any fish or shellfish species (Faruque *et al*, 2010). However, the larval rearing of *O. belangeri* is still not standardised. Development or identification of suitable feed resources which are accepted and utilized well by the species of choice is vital for growth and survival of their larvae (Mohseni *et al,* 2012). In this regard, it is remarkable that fish larvae have tiny mouth gaps and are highly fragile, and have rudimentary sensory and digestive system.

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These morphological and physiological conditions put severe restrictions on nutritional contents, quality, size and other characteristics such as floatability of suitable larval feeds (Kolkovski *et al*, 2009, Hamre *et al*, 2013). These limitations are often overcome by utilizing live feeds and/or undertaking larval rearing in fertilised ponds where natural foods, including phytoplankton and zooplankton, are abundant. Artificial feeds such as mixture of rice bran and oil cake play only a supplementary role in pond culture systems. Rathod *et al* (2014) evaluated Moina with or without enrichment and artificial micro-encapsulated (crude protein 46%) feeds in different combinations for *O. belangeri* larvae in clearwater system and observed superior performance with live feeds and/or co-feeding with Moina and artificial feeds while markedly poor survival and growth performance with artificial micro-encapsulated feed.

In this context, it is notable that high proportions of aquatic macrophytes have been observed in the guts of juvenile *O. belangeri* caught from open ecosystem indicating natural preference of aquatic algae. Therefore, it may be of relevance to evaluate the relative efficacy of amended aquatic phyto-origin feed resources as a source of nutrition for *O. belangeri* larvae. Accordingly we considered *Wolffia globosa* (henceforth referred as Wolffia), a rootless and the smallest (size: 0.3-0.6 mm) flowering plant on the earth, to be a potential valuable single-ingredient allochthonous feed based on many desirable nutritional attributes including high contents of crude protein (25-40%) of favourable essential amino-acid profiles, high minerals, vitamins and bioactive compounds like carotenoids and flavonoids, and low content of fibre (10-15%) and anti-nutritional factors (Leng *et al*, 1995). Further, live Wolffia has also been demonstrated as an excellent allochthonous single-ingredient feed for the rearing of 20-d old fry of *Labeo rohita* (Pradhan *et al*, 2019).

However, it is not known if live Wolffia could be an effective and efficient live feed in crushed form or dried meal form for one week old larvae of *O. belangeri*. One important apprehension was that crushing fresh Wolffia for making it amenable to ingestion by pengba larvae may lead to substantial losses of nutrients due to cell rupture and consequent loss of cellular milieu. On the other hand, the dry meal of Wolffia while retaining most of the nutritional attributes may lose excellent floatability and bioactive components. The present study attempted to evaluate the relative efficacy of Wolffia in crushed-live and dry meal forms as a sole allochthonous nutritional source for 7-dph *O. belangeri* larvae in clearwater system.

MATERIALS AND METHODS

Experimental fish

The 7-dph larvae of *O. belangeri* (mean weight 2.00±0.04 mg; mean length 5.50±0.50 mm) were obtained from college farm of College of Fisheries, CAU (I), Lembucherra, Tripura (W), India. Larvae were carefully stocked into 12 uniforms sized glass aquariums (60 cm×30 cm×30 cm) filled with 40-L of conditioned groundwater at a stocking density of 3 larvae L^{-1}, *viz.* 120 larvae per aquarium and each aquarium was provided with aeration. The nursing was carried out for three-weeks, *viz.* 21 days.

Feeds and feeding

Four different feeds namely, a simple powder mixture of mustard oil cake and rice bran (D1; Crude protein 19%), a multiple ingredient-based compound feed consisting of rice bran, mustard oil cake, soybean meal, fish meal and, vitamins and minerals (D2: 29%); crushed fresh Wolffia (D3; Crude protein 21%) and; a dry meal Wolffia (D4; 29%) were utilised. The ingredient compositions of experimental feeds are presented in Table 1. Larvae were acclimated in the experimental units for two (2) days during which they were fed with mixed populations of zooplankton collected from nearby productive earthen ponds in the college farm. After two days, experimental feeds (D1-D4) were applied randomly to the aquaria in triplicates. The larvae were fed three times a day (8.00 AM, 12.00 PM and

Table 1: Ingredient composition (%) of experimental feeds on dry matter basis.

Name of Ingredient	Composition in Experimental Feed (%)			
	D1	D2	D3	D4
Rice bran	50	35	-	-
Mustard oil cake	50	20	-	-
Soybean meal	-	20	-	-
Fishmeal	-	20	-	-
Vitamin + Mineral mixture	-	5	-	-
Crushed Fresh Wolffia	-	-	100	
Dry meal of Wolffia	-	-	-	100

4.00 PM) at the combined feeding rate @100%-200% bodyweight d^{-1} by dividing the feed ration equally at each occasion.

Water quality analysis

Water quality parameters, including temperature, pH, total ammoniacal nitrogen (TAN), nitrite-N, nitrate-N, and orthophosphate (PO_4-P) were monitored weekly. The pH and temperature were measured with digital pH meter (HI 991001, HANNA). TAN, nitrite-N, nitrate-N and orthophosphate (PO_4-P) in water were measured by Continuous Flow Analyser (SA3000/5000, SKALAR) equipped with auto-sampler (SA 1100, SKALAR) based on standard methods. In summary, water samples were collected from each aquarium and filtered with GF/F (Whatman glass fibre filter) within 30 minutes of collection. The filtrates were collected and stored in clean polypropylene sample bottle (50-100 ml) at -20°C in the freezer, till analysed.

Statistical analysis

The data processing was done using MS Excel, and statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 16.0 for windows). One-way Analysis of Variance (ANOVA) was performed to determine the significant differences between treatment means. In case treatment effects were significant, Duncan's New Multiple Range test at $p<0.05$ level (Duncan, 1955) utilised to rank treatments.

RESULTS AND DISCUSSION

General water quality parameters

General water quality parameters, including temperature, pH, TAN, nitrite-N, nitrate-N, and orthophosphate (PO_4-P) remained within the normal range during the entire rearing period. The pH of water ranged between 7.32-8.01, which was close to the optimal range for tropical fish culture (Akinwole and Faturoti, 2006). The TAN, nitrite-N, nitrate-N and orthophosphate of water were low during the entire culture period and were close to the optimal reported values by Boyd and Lichtkoppler (1979).

Growth performance and survival

The final mean weight and final mean length of *O. belangeri* larvae showed highly significant ($p<001$) variations between different treatments (Table 3). The highest final mean weight (73.70±1.53 mg) and final mean length (12.83±0.44 mm) were observed for D2 followed by those for D1 (53.37±3.07 mg and 11.67±0.17 mm), D4 (46.03±2.11 mg and 10.17±0.33 mm) and D3 (27.87±0.58 mg and 9.83±0.44 mm), respectively in order (Table 2). Thus, the final mean weight of larvae fed on multiple ingredient-based compound feed (D2) was 1.5-2.5-folds higher than those of rest. Correspondingly the specific growth rates also varied significantly ($p<0.001$) among treatments with the highest values for D2 and the lowest for D3 with D1 and D4 showing intermediate values.

The superior growth performance of *O. belangeri* larvae with multiple ingredient-based compound feed (D2) over D1 was in synchrony with superior nutritional attributes of the former, especially with respect to protein and vitamin and mineral contents. The mean survival of the *O. belangeri* larvae also showed marked variabilities and ranged between 29.7% (D3) and 61.9% (D2) with intermediate values for D4 (55.8%) and D1 (53.3%). Thus, in general, treatments exhibiting superior growth performance also showed relatively higher survival. However, notably, no significant differences ($p>0.05$) in survival between treatments were established statistically.

The general obtained growth and survival of a week old (7-dph) *O. belangeri* larvae in the present study were within the reported range of survival rates for carps, and mostly superior to that reported for *O. belangeri* by Rathod *et al* (2014). For that matter, Rathod *et al* (2014) reported survival of *O. belangeri* larvae between 37.2%-62.66% with live feed (Moina with or without enrichment with cod liver oil or algae) and artificial micro-encapsulated feed (crude protein content 46%) or co-feeding with the two. While they obtained significantly better growth performance of *O. belangeri* larvae with the combination of enriched Moina and micro-particulate feed than the other groups while the growth and survival with micro-encapsulated feed was the lowest. The survival was low as 38% which is remarkably lower than those obtained in the present study with multiple ingredient-based compound feed (D2; 62%) and a simple mixture of rice bran and mustard oil cake (D1; 53%). It is also relevant to point out that the crude protein contents of D1 (19%) and D2 (29%) were remarkably lower in comparison to that of micro-encapsulated feed utilised by Rathod *et al* (2014). Similarly, the growth performance obtained in the present study is also superior to those observed by them. Thus, the results indicated that even a week-old (7-dph) *O. belangeri* larva could ingest and metabolise simple feed mixture of rice bran and oil cakes as well as a more complex multiple ingredient-based compound feed reasonably well. That, *O. belangeri* larvae could utilise a simple mixture of rice bran and mustard oil cake rather satisfactorily for fulfilling nutritional needs in the absence of natural foods was somewhat surprising. For that matter, Mohseni *et al* (2012) also reported that one (1)-week old beluga (*Huso huso*) larvae could not utilise formulated feed well and exhibited relatively higher mortality. Keer *et al* (2018) has reported that survival of *Cirhinus reba* during spawn to fry nursing (outdoor) between 46.85-55.80%, which was closer to our present study despite the absence of natural food during the present experiment. Similar findings had been corroborated by Canavate *et al.* (1999) and Kolkovski (2001).

Considering that effectiveness of larval feeds is positively linked with feeding behaviour of larvae, it is essential to delineate some of the behavioural observations of *O. belangeri* larvae as well as specific changes in rearing systems of different treatments. In this regard, it was most notable that the water of aquaria recipient of D3, *viz.* crushed fresh Wolffia turned bright greenish during the early phase of the experiment. Further, larvae in these turned-greenish aquaria were visibly smaller and less active as compared to those of other aquaria. The inferior growth and survival of *O. belangeri* larvae for D3 might have been results of direct loss of nutrients caused by a cell rupture. For that matter, the crude protein content of crushed fresh Wolffia (analysed after drying) was only 21% against 29% in dry meal of wolffia. This implied that about 30 % loss of protein due to crushing. Furthermore, the microscopic analysis had indicated that crushing did not result in uniform-sized particles and sizes of crushed wolffia ranged from <0.2 mm to about 6.0 mm implying that despite crushing a substantial portion of Wolffia was probably too large to be consumed by larvae of less than 10 mm size. On the other hand, it could also be possible that the predominance of cell walls that are rich in starch and cellulosic materials, in D3 might have been less

Table 2: Ranges of different physicochemical parameters of water for different treatments during the culture period.

Parameter	Treatment			
	D1	**D2**	**D3**	**D4**
pH	7.32-7.51	7.61-7.90	7.42-7.74	7.79-8.01
Temperature (^{0}C)	28.6-30.3	28.4-30.3	28.4-29.5	28.3-29.6
Ammonia-N (mg l^{-1})	0.60 – 0.75	1.03 – 1.41	0.18 – 1.06	0.13 – 1.03
Nitrite-N (mg l^{-1})	0.02 – 0.09	0.03 – 0.11	0.31 – 0.59	0.19 – 0.57
Nitrate-N (mg l^{-1})	0.01 – 0.06	0.01 – 0.02	0.01 – 0.11	0.03 – 0.08
Orthophosphate-P(mg l^{-1})	0.21 – 0.34	0.30 – 0.51	0.13 – 0.15	– 0.48

amenable to digestion by carp larvae as indicated by Segner *et al*. (1993). On the contrary, D1 and D2 exhibited superior floatability of D1 and D2 in comparison to D4. The dry meal of Wolffia (D4) has markedly high specific density and sank to the bottom of the aquarium rather rapidly in comparison to D1 and D2. This probably rendered D4 unavailable to *O. belangeri* larvae pretty quickly which mostly occupied water column and surface water and leading to relatively poor growth and survival of pengba fry.

The right balance of nutritional contents and quality of larval feed has been well known to be crucial factors in impacting growth and survival. In this regard, D2, which had more favourable proportions of nutrients, particularly in comparison to D1, led to most superior growth performance and survival. More specifically, D2 exhibited 1.5-fold higher growth performance and 1.2-fold higher survival in comparison to a simple conventional mixture of rice bran and mustard oil cake (1:1; D1). Considering that higher growth has distinct advantages over smaller counterparts and also dramatically reduces the chances of mortality in the subsequent phase of life, use of D2, *viz*. a multiple ingredient-based compound feed is recommended. Nevertheless, it is also to be emphasised that a simple mixture of rice bran and mustard oil also was an effective feed even for 7-dph *O. belangeri* larvae even in the absence of natural foods. Similarly, dry meal of Wolffia single-ingredient feed may also be an effective larval feed for 7-dph *O. belangeri* larvae. Further trials may be conducted with multi-ingredient based compound feed and/or simple mixture utilising dry Wolffia meal in particular whereby taking care to reduce the specific gravity in particular.

Table 3: Growth performance and survival of *Osteobrama belangeri*

Parameter	Treatment			
	D1	**D2**	**D3**	**D4**
Initial mean weight (mg)	2.00±0.04	2.00±0.04	2.00±0.04	2.00±0.04
Final mean weight (mg)	53.37±3.07^c	73.70±1.53^d	27.87±0.58^a	46.03±2.11^b
Initial mean length (mm)	5.50±0.50	5.50±0.50	5.50±0.50	5.50±0.50
Final mean length (mm)	11.67±0.17^b	12.83±0.44^b	9.83±0.44^a	10.17±0.33^a
SGR (% day $^{-1}$)	15.62±0.27^c	17.17±0.10^d	12.54±0.10^a	14.92±0.22^b
Survival (%)	53.33±14.25	61.94±16.89	29.72±3.20	55.83±6.61

Data shown in the table were Mean ± SE, n = 3. The mean values with different alphabetical superscript within a row for parameters were significantly different (P<0.05).

CONCLUSION

The results of the present study indicated that 7-dph *O. belangeri* larvae could utilise artificial feeds rather effectively. The multiple ingredient feed enriched with vitamin and minerals (D3; crude protein 29%) resulted in 1.5-fold higher final mean weight and 1.2-fold higher survival than the second-best performing feed, *viz.* a simple mixture of rice bran and mustard oil cake (crude protein 19%). The *O. belangeri* larvae exhibited the lowest survival and growth performance with crushed fresh Wolffia (D3), indicating its general unsuitability. The nutrient loss due to mechanical rupture and non-uniform sized particles caused by mechanical crushing of live Wolffia apparently were the main constraints in its utilisation. The dry meal of Wolffia (D4), on the other hand, had higher specific gravity and sank rather quickly impacting its ingestion by the *O. belangeri* larvae which mostly occupied water column and surface water. Further studies with intact live wolffia where nutrient content, water stability and floatability of live wolffia is not compromised is required. Further, homogeneous mixture of finely grounded dry wolffia meal with other ingredients to improve its general floatability may also be explored.

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