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A Comparison of Artificial and Natural Diets for Growth and Survival of Stinging Catfish *Heteropneustes fossilis* Larvae

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ABSTRACT

The present experiment was conducted to evaluate growth performances and survival of stinging catfish, *Heteropneustes fossilis* larvae fed different larval diets, viz., artificial diet, egg custard, live *Artemia* nauplii (LAN), frozen *Artemia* nauplii (FAN), and wild mixed zooplankton (WMZ). Induced bred larvae (5- day old) of stinging catfish were fed on the experimental diets for 21 days. The larvae-fed LAN showed significantly the highest growth performances (Average final weight= 475.2 mg; specific growth rate =28.37% day^{-1}). In addition, WMZ showed better growth performances (Average final weight of 204.4 mg; the specific growth rate of 24.35% day^{-1}), compared to the artificial diet (Average final weight= 40.4 mg; specific growth rate= 16.62% day^{-1}). While, no growth performance was detected for FAN (Average final weight of 192.1 mg; the specific growth rate of 24.05% day^{-1}), compared to LAN. Egg custard showed poor growth performances (Average final weight= 110.7 mg; specific growth rate= 21.43% day^{-1}); however, still better than the artificial diet. The survivability of larvae was affected by the diets supplied and exhibited a pattern similar to that of the growth performances. Therefore, based on the results, LAN was the best for feeding stinging catfish larvae. However, as the performance of WMZ was better than the artificial diet and considering the high cost of *Artemia* cysts, WMZ can be recommended as a cost-effective larval feed for stinging catfish.

INTRODUCTION

Heteropneustes fossilis **(Bloch, 1974)**, commonly known as stinging catfish, belongs to the family Heteropneustidae and the order Siluriformes. This species is a popular catfish and commercially important in Asian aquaculture, particularly in countries like Bangladesh, Thailand, India, Pakistan, Nepal, Sri Lanka, Myanmar, Indonesia and Cambodia **(Rahman *et al.*, 2013; Kumar *et al.*, 2018)**. Stinging catfish is considered an ideal fish species for aquaculture because of its fast growth, tolerance to high stocking densities, high market value, ability to survive in oxygen-low waters, low fat, high protein, iron content, and medicinal values **(Puvaneswari *et al.*, 2009)**. However, the culture of this catfish hasn't increased due to the non-availability of a sufficient quantity of seed because of high mortality rates during larval rearing and a lack of knowledge of larval feeding strategies and rearing techniques. Therefore, effective and reliable larval rearing methods must be developed for the successful culture of this catfish species to ensure a consistent supply of seed. The induced spawning technique of stinging catfish is successful; nevertheless, there are still problems with larval rearing **(Rahman *et al.*, 2013).** Eminently, the absence of a readily available starter feed in commercial hatcheries remains a major problem in its production. The larval stage of fish is considered the most sensitive phase of its life. Fish larvae rely on the yolk sac for their nutritional requirements during the early stages of growth. Then larvae require exogenous food. It has been shown that formulated compound diets do not provide optimal larval growth when used exclusively as larval food, especially during the early larval stages of cyprinids and catfish **(Santhanam *et al.*, 2004; Martin *et al.*, 2006; Mostary *et al.*, 2007).** Although some freshwater fish larvae can be fed formulated diets as early as mouth opening, the live feed is as a rule substituted with compound diets only after some weeks of life in marine fish in the hatchery **(Cahu & Infante, 2001)**. However, the early introduction of formulated diets as the sole feed source has met limited success, even in freshwater species **(Sales, 2011).**

As exogenous food, live foods such as *Artemia* nauplii, yeast, unicellular algae, rotifers, copepods, and cladocerans are the most appropriate starter foods since the larvae have difficulty in assimilating dry prepared diets due to their incomplete development of the digestive system **(Arimoro, 2006; Hossain *et al.*, 2007; Yoshimatsu & Hossain, 2014)**. These live foods offer an appropriate size ingestible by a wide range of larval fish species and are rich carriers of digestive enzymes **(Kolkovski, 2001; Rasdi *et al.*, 2016)**. Live foods also affect the fatty acid profile of larvae **(Das *et al.*, 2007; Tocher, 2010)**. In this context, because information on the appropriate feeds for the larviculture of stinging catfish is scarce, the present study was undertaken to compare some artificial and natural diets for the larval rearing of stinging catfish.

MATERIALS AND METHODS

Artificial Diet

The artificial diet containing 40 % protein was collected from a local fish feed manufacturer (Mega Feed Ltd.). The feed was made of fishmeal, maize, rice polish, mustard oil cake, soybean meal, flour, meat and bone meal, salt, dicalcium phosphate, and vitamin-mineral premix, designated as catfish starter feed. The proximate composition of the artificial diet, as analyzed in the laboratory, is shown in Table (2).

Collection and Identification of Wild Mixed Zooplankton (WMZ)

WMZ was collected from a lake inside Bangabandhu Sheikh Mujibur Rahman Agricultural University Campus. The lake was eutrophic due to a wastewater drain, where an abundance of rotifers was observed in the initial sampling. WMZ were collected from a depth of 10-15cm below the water surface using a plankton net (120 µm). The WMZ was dominated by rotifers, followed by cladocerans and copepods (Table 1). The collected zooplankton samples were identified using standard manuals **(Murugan *et al*., 1998; Altaff, 2004; Kumar, 2015)**.

Table 1. Composition of WMZ used as diet in the experiment

Zooplankton group	Species	Composition (%)
Rotifer	*Asplanchna* sp. *Brachionus calyciflorus* *Brachionus falcatus* *Brachionus angularis* *Keratella* sp. *Filicia* sp.	52.3
Cladoceran	*Diaphanosoma* sp. Moina *micrura* *Moina brachiata* *Daphnia pulex* *Daphnia magna*	21.8
Copepod	*Cyclops Vernalis* *Eucyclops separatus* *Diaptomus* sp.	12.2
Others		13.7
	Total	100

Table 2. Proximate composition of experimental diets

Composition	Artificial diet	Egg custard	LAN	FAN	WMZ
Moisture (%)	10.83	-	90.09	90.01	93.05
Crude protein (% d.m.)	38.27	38.05	57.33	57.42	39.23
Crude lipid (% d.m.)	9.60	18.70	13.45	13.52	19.45
Crude ash (% d.m.)	11.35	10.61	6.23	4.40	5.93

d.m. = dry matter.

Live and Frozen *Artemia* Nauplii

Artemia nauplii were obtained from cysts (OSI Brine Shrimp Eggs, USA) incubated in the hatching jars containing tap water with added NaCl to give a salinity of 15 ppt (3.0 g *Artemia* cysts in 4 L water). After 20-24 hours of incubation at 28^{o}C, the nauplii were separated from cyst shells and collected. A portion of the *Artemia* nauplii was fed to the larvae as live *Artemia* nauplii (LAN). The remaining nauplii were preserved at -18^{o}C in a refrigerator until used as frozen *Artemia* nauplii (FAN).

Egg Custard

The ingredients of egg custard (60% egg and 40% powdered milk) were mixed by a blender and boiled for 30min to make a cake. Then, the egg custard was passed through a small mesh net, and the collected small particles were washed in water to remove the soluble materials. The particle-sized egg custard was adjusted as the larvae grew up. The egg custard was daily prepared and kept refrigerated at 4°C until use.

Stocking and Rearing of Larvae

Newly hatched larvae of stinging catfish were collected from a fish seed hatchery at Trishal, Mymensingh. The larvae were transported in an oxygenated polythene bag and kept in a tank while fed with egg custard. After 5 days, a total of 100 larvae (average weight of 1.23mg) were transferred to a plastic container containing 20-liter of water. There were fifteen larval rearing containers for 5 different diets, each with 3 replications. The larvae were fed the experimental diets viz., commercial diet, egg custard, LAN, FAN, and WMZ ad libitum three times per day during the 21 days trial. The concentrations of the live feed (FAN and WMZ) in the respective containers were adjusted depending on the residual live feed concentration in the containers. Wastage accumulated in the bottom of the containers was cleaned every day in the morning by siphoning. Every day, about 30-40% of the water in the container was exchanged. Water quality parameters such as water temperature, dissolved oxygen, and pH values were 28.2–29.1°C, 5.7 –6.5 mg l^{-1}, and 7.1–7.6, respectively, during the experiment period.

Sampling

Stinging catfish larvae were inspected every day to check mortality through visible inspection. Larvae were sampled at the end of 3 weeks to observe their growth rate and survival rate. Water quality parameters viz., temperature, pH, and dissolved oxygen were monitored regularly with a Celsius thermometer (Digi-Thermo WT-2), digital pH meter (Hach Co., Colorado, USA), and an oxygen meter (Hach Co., Colorado, USA), respectively.

Proximate Analysis of Sample

The proximate compositions of the experimental diets were determined according to the standard method set by the Association of Official Analytical Chemists **(AOAC, 2000)**. Briefly, crude protein content was determined using the Kjeldahl systematic method following acid digestion with an auto-Kjeldahl System (UDK 152, VELP); crude lipid content was assessed by ether extraction using a solvent extractor (SER 148, VELP);

crude ash was measured following combustion at 550°C, and moisture content was detected by drying the samples in an oven at 105°C for 24 h.

Growth and survival rate of larvae

At the end of 21- day rearing period, the final weight of larvae was recorded, and the total number of larvae in a container was counted. Then, specific growth rate and survivability were counted as follows:

Specific growth rate (% day^{-1}) = 100 [Ln W2 (g) − Ln W1 (g)] / T, where W1 = initial fish weight; W2 = final fish weight, and T is the rearing period (21 days);

Survival rate (%) = (Total number of fish at harvest / Total number of fish at stock) × 100

Statistical Analysis

All the data were collected, recorded, and preserved during the experimental period on a computer spreadsheet. The data were statistically analyzed by one-way ANOVA using statistical software statistics 10 (2013); significance was indicated by the Least Significant Difference (LSD) option of the package, which was used to compare the means. The significance level was determined at $P<0.05$.

RESULTS

The weekly growth of stinging catfish larvae is shown in Fig. (1). The initial average weight of larvae of all diet groups was 1.23g. The larval growth was rapid, where LAN was used as food from the beginning of the experiment, and the trend continued until the end of the 21- day trial. In other diet groups, growth was initially slow and started to increase beginning from the end of the 1st week. At the end of 21- day trial period, the highest average final weight (475.2 mg) in the larvae fed on LAN was significantly higher than the larvae fed an artificial diet (40.4 mg). Average final weights of larvae fed WMZ (204.4 mg) or FAN (192.1 mg) were lower than larvae fed on LAN; yet, still greater than larvae fed an artificial diet or egg custard (110.7 mg).

The highest SGR was observed in fish fed with LAN (28.37 % day^{-1}) followed by WMZ (24.33 % day^{-1}), FAN (24.05 % day^{-1}), egg custard (21.43 % day^{-1}), and artificial diet (16.63 % day^{-1}), respectively (Fig. 2). Acceptability of FAN to larvae was lower than LAN due to non-movability. SGR of larvae fed with WMZ was lower than larvae fed on LAN; however, it was still better than larvae fed with an artificial diet or egg custard. Although feeding egg custard showed better SGR values (21.43 % day^{-1}) than the artificial diet, it was lower than the other three diets.

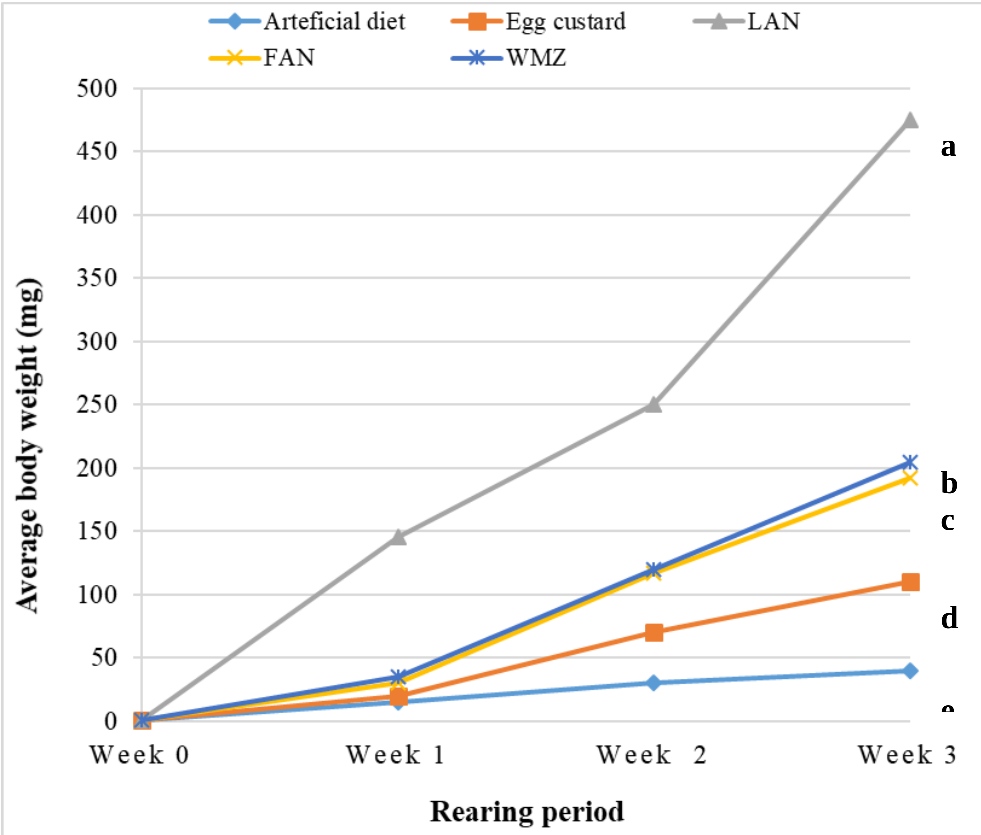

Fig. 1. Average body weight of stinging catfish fed on experimental diets

Different letters indicate significant difference (*P*<0.05).

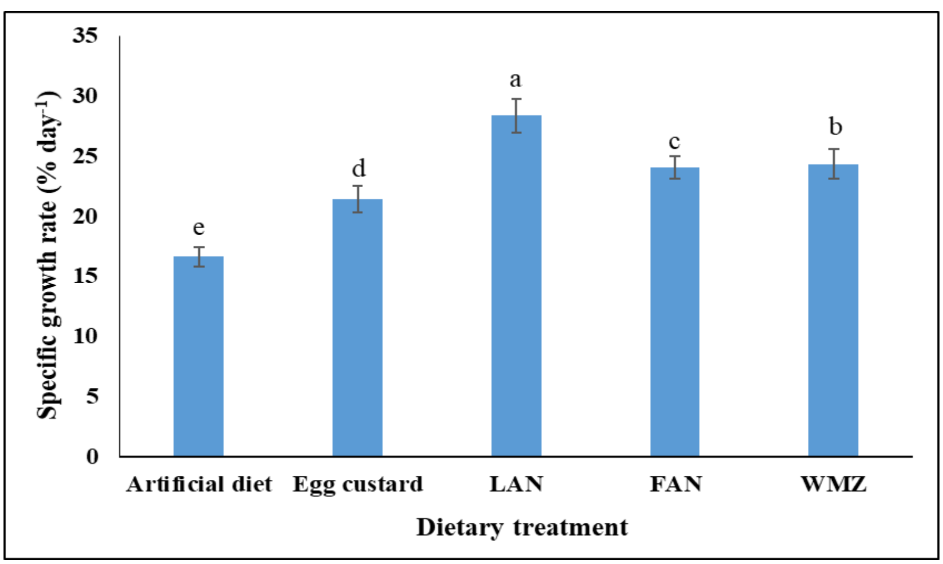

Fig. 2. Specific growth rate (% day^{-1}) of stinging catfish fed five different diets

Different letters indicate significant difference (*P*<0.05).

The survival rates were 92.2, 84.1, 81.7, 74.3, and 64.7 in larvae fed on LAN, WMZ, FAN egg custard, and artificial diet, respectively (Fig. 3). The highest survival rate was recorded in larvae fed on LAN. The survival rate of larvae fed on WMZ or FAN was similar and lower than those fed on LAN though still better than larvae feed egg custard or artificial diet. Survivability was lower in larvae fed egg custard or artificial diet, and the poorest survival rate was observed in larvae fed on artificial diet.

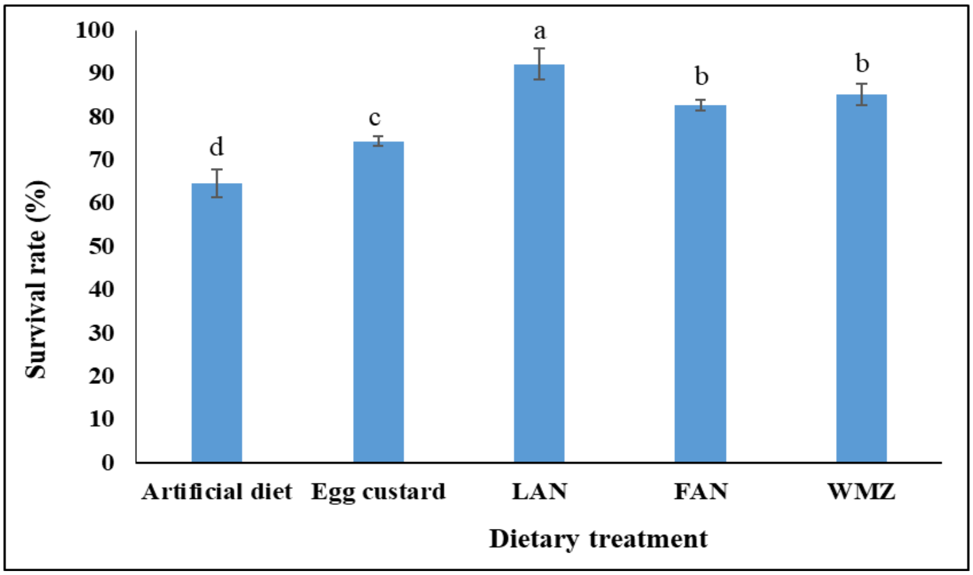

Fig. 3. Survival rate (%) of stinging catfish fed with five different diets. Different letters indicate significant difference ($p<0.05$).

DISCUSSION

The nutrition of fish larvae, both qualitatively and quantitatively, may differ from those of juveniles or adult fish since fish undergo dramatic morphological and physiological changes, including metamorphosis, during ontogenesis. Moreover, fish larvae grow extremely rapidly, feed continuously, and, therefore, the total ingestion of nutrients must be high **(Hamre *et al.*, 2013)**. It is evident from the present study that the stinging catfish larvae more effectively utilized live food organisms than the dry artificial diet. Live food organisms contain all the nutrients such as proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty acids. Hence, live feeds are popularly known as "living capsules of nutrition" **(Manickam *et al.*, 2017)**. **Shourbela *et al.* (2005)** observed that the African catfish *Clarias gariepinus* larvae utilized the live organisms more efficiently than the artificial diet. The live feed fed fish and crustacean larvae exhibited better survival and growth rate when compared to the artificial diets **(Santhanam *et al.*, 2004; Evangelista *et al.*, 2005)**. In larval rearing of a freshwater catfish, *Ompok bimaculatus*

(Malla and Banik, 2015) observed higher SGR in the fish feed fed with live feed than compound feed. Various researchers have attributed this phenomenon to the presence of enzymes in live feeds that hasten the digestion and absorption processes in first -feeding larvae **(Kolkovski, 2001; Radhakrishnan *et al*., 2020).**

In the present study, regardless of time, LAN-fed larvae had consistently high growth, and the survival rate also was the highest in this group. These findings agree with an earlier report on Asian Catfish, *Pangasius bocourti*, where LAN was excellent food for larvae **(Hung *et al.*, 2002). Demir *et al.* (2014)** also justified the best growth and survival for the catfish *Rhamdia queen* during the first 15 days of feeding with LAN that offer an advantage in larvae weaning, to consume artificial food. **(Faruque *et al.*, 2010)** carried out research on the growth performance and survival of African catfish *(Clarias gariepinus)* larvae and found that larvae tend to use Artemia more efficiently than artificial food. The high performance of LAN could be due to higher level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and other polyunsaturated fatty acids (PUFA) that are essential for the growth and health of fish larvae **(Rasdi *et al.*, 2016)**. The suitability of LAN as food for catfish larvae could also be because of their very high digestibility. Artemia contains quite high levels of water-soluble protein (approximately 50%), which is more digestible than insoluble protein **(Carvalho *et al.*, 2003; Tonheim *et al.*, 2007),** making the protein in live prey more bioavailable than that in artificial diets.

FAN has been considered suitable for larval rearing as it can be stored and can be used readily. However, the performance of FAN was lower than LAN in the present study. Because the suitability of live foods depends on their ability to swim in the water column and are constantly available to finfish and shellfish larvae that are likely to stimulate larval feeding response **(Conceicao et al., 2010)**, this attribute is absent in FAN. In research with the species *Litopenaeus vannamei*, frozen umbrella stage *Artemia* favors the growth and survival of the larvae **(Cobo *et al.*, 2015)**. However, comparing the *Artemia* nauplii with the frozen nauplii, this last one did not favor the survival of the *L. alexandri* larvae, probably because they lack nauplii motility that did not attract catfish larvae. According to **Hamre *et al.* (2013)**, some species are guided by the vision to feed, detecting live food more easily due to movements.

The performance of WMZ was superior to artificial diets. The WMZ has higher digestive enzyme contents, and therefore, they can be used by the fish larvae as exo-enzymes **(Gangadhar *et al*., 2018).** Especially, WMZ contains a high level of nutrition profiles such as biochemical (protein, carbohydrate, and lipid), digestive enzyme (protease, amylase, and lipase), non-enzymatic antioxidant (Vitamin C and E), profiles of amino acid and fatty acid **(Rasdi *et al*., 2016; Manickam *et al.*, 2017, 2020).** Zooplankton has been considered good for fish larvae. The juveniles of the cultivable species can get their nutritional requirement, as they show it has higher nutritional value; hence it plays a vital role in initial feeding for survival and growth **(Rajkumar *et al.*, 2004; Samat *et al*.**

2020). However, the growth performances of stinging catfish fed WMZ were lower than that of the larvae fed on LAN. The WMZ was dominated by three groups such as a Rotifera, cladoceran, and Copepoda. Some of the zooplankton in WMZ may have been too small (150–250 µ); thus, the stinging catfish larvae must have spent more energy seeking their food**. Pradhan and Barman (2014)** found superiority of LAN over mixed WMZ in the case of a catfish *Ompok bimaculatus*.

Poor growth and survival of stinging catfish larvae given artificial feed proved that prepared diets are not yet suitable for first feeding larvae. The low ingestion rate of artificial feeds and leaching nutrients from dry feeds have been highlighted as factors behind the poor growth in fish larvae fed dry feeds **(Muguet *et al.*, 2011)**. **Fermin and Bolivar (1991)** and **Evangelista *et al. (*2005)** reported that the specific growth rate of *Clarias macrocephalus* larvae fed live feed was higher than those fed non-live feed. Although egg custard was appeared to be better than that arterial diet in the case of the present study; however, it was not comparable to other diets. Similarly, in the case of *Macrobrachium rosenbergii*, it was observed that the total replacement of *Artemia* sp. with egg custard is not sufficient to support the growth and survival of the prawn at stage VI, probably because some larvae are not ready to make egg custard alone as their sole source of food and still preferred the live feed **(Shailender *et al.*, 2012; Nik Sin and Shapawi, 2017)**. **Sarkar *et al.* (2006)** also obtained poor performance of egg yolk compared different live feeds in chital *Chitala chitala*. Furthermore, they reported that egg-yolk broke rapidly in water lead to an increased fouling rate. Therefore, the least growth performances observed in larvae exclusively fed egg custard could be attributed to a less developed digestive system that cannot fully support fish larvae digest artificial dry feed or egg custard.

Based on the results of this experiment, it is clear that the larval rearing of stinging catfish using LAN can be applied for the highest growth and survival rate. However, the biggest disadvantage of Artemia has been the marked variation in price. Therefore, although the FAN is not so attractive, it still presents the process of autolysis that assists in its assimilation by the larvae. It should also be considered that FAN is feasible and practical due to the possibility of storing and being offered when the production of living organisms fluctuates **(Nascimento *et al.*, 2020)**. Already, the FAN was a viable food but should be used preferably in times of lack of live nauplii. WMZ is available at minimal cost, although seasonal variation in species composition can be a concern. However, WMZ can be a good choice for a farmer, considering the high cost and availability of Artemia cysts.

CONCLUSION

Artificial diet and egg custard are not suitable as larval feed for stinging catfish. Among the live feeds, LAN showed the best performance. However, FAN did not find attractive to stinging catfish larvae as LAN. However, still was better than the artificial diet and can be used when LAN if not available or preparation of LAN daily is troublesome. WMZ showed inferior growth performances compared to LAN; however, superior to artificial diet or egg custard. Considering the high cost of Artemia cysts, WMZ can be considered as a cost-effective feed for larvae of stinging catfish.

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