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Effect of Live and Formulated Feeds on Larval Growth and Survival of Guppy (*Poecilia reticulata*) Reared in Indoor Tanks

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ABSTRACT. Two live feeds, Artemia nauplii (AN) and Anguillula silusae, known as microworm (MW), and three formulated feeds varying in their source of lipid i.e., cod-liver oil (A1), soybean oil (A2) and coconut oil (A3) were tested with guppy larvae for 20 days covering the entire larval period. Along with these diets culture medium (CM) of MW was also included as a test diet. Each treatment/test diet had three replicates and the unfed control (UC) had two replicates. Water quality was maintained by regular daily water exchanges. Survival was 75% or more at the end of the feeding trial except for UC where survival was 0%. The test diet AN resulted in significantly higher (p<0.05) growth, in terms of larval length, weight and specific growth rate (SGR) than all other diets tested. There was no significant difference (p>0.05) among fish fed with diets A1, A2 and A3 on the above trails. This suggests that costly cod-liver oil can be replaced with cheaper soybean or coconut oil in formulated feed for guppy. The results indicate that AN is the best diet tested in this study. However when production costs are considered MW is a much cheaper feed that results in satisfactory larval performance.

INTRODUCTION

Ornamental fish culture is a high income generating practice when economic return per fish is considered. The value of total exports of ornamental fishes from Sri Lanka, which was 93 million rupees in 1991, increased up to 530.7 million rupees in 1998 (NARA, 1999). This increase was mainly attributable to development in technology, both in capturing and culturing fish. Presently there are three large-scale exporters and about 40 medium and small-scale exporters (Edirisinghe, 1999). One of the major strengths and prospects of bright future of Sri Lankan ornamental fisheries sector is that Sri Lanka, being located in the centre of the vast indo-pacific faunal region, has a unique and diverse composition of both freshwater and marine fish species. Also Sri Lanka has both the necessary climatic and water resources required to develop successful production of many species of freshwater ornamental fishes and plants. Nevertheless comparatively little research effort has been paid to this sector.

Several drawbacks have been identified in Sri Lankan ornamental aquatics sector. Understanding and awareness of fish, particularly larval fish nutrition among ornamental fish farmers are poor (Mee, 1993). Furthermore imported cyst forms of live feeds are costly to the small and medium-scale ornamental fish farmers in Sri Lanka. This necessitates the search for new live feeds. The nematode worm *Anguilulla silusae*, commonly known as microworm (MW) is a promising larval feed. Meanwhile low cost formulated feeds that

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results in satisfactory growth in fish larvae should also be developed. One possible way to achieve this is to cut down the production cost by replacing marine oils with terrestrial plant oils. Development of low cost feeds is important especially to guppy, *Poecilia reticulata*, which constitutes 60% of the total exports by number (Edirisinghe, 1999).

Hence the objectives of this study were to develop low cost formulated feeds and compare them with the common live feed *Artemia* nauplii (AN), MW and its culture media (CM) in terms of total length, wet weight, specific growth rate (SGR) and survival of guppy for 20 days covering the entire larval period.

MATERIALS AND METHODS

Preparation of test diets

Artemia nauplii (AN) were obtained by hatching *Artemia* cysts (origin: Great Salt Lakes, Utah, USA) following the method described by Bengston *et al.* (1991). Here an adequate amount of salt was added to tap water to increase the salinity up to about 28 ppt. When cysts hatched after 24 h, nauplii were sieved through a 150µ mesh and those trapped were used for feeding.

Microworms (MW) were cultured and separated using the method described by Hirimuthugoda *et al.* (1999). The third test diet, the microworm culture media (CM) was a mixture of bread and soybean powder at a ratio of 9:1. CM was used in the experiment in order to determine the nutritional superiority of MW relative to the CM.

The ingredients commonly used by farmers were used in different combinations to develop the three formulated diets. Cod-liver oil (commonly used by farmers), soybean oil and coconut oil were used as sources of lipid in formulating the diets A1, A2 and A3, respectively. The compositions of these diets are given in Table 1. Dry ingredients were mixed thoroughly and water was added adequately to make a thick paste and then dried in an oven at 60°C for 24 h. The dried feed was then ground with a grinder and sieved to obtain fine particles. The prepared feeds were stored in a refrigerator.

Proximate analysis

A proximate analysis was conducted to determine the nutritional quality of all six test diets used in the experiment. Crude protein was determined by the Kjeldahl method (N×6.25) (Tecator Kjeltec System, 1026 Distilling Unit). Fat content was determined by Sohxlet extraction method. Ash content was determined by the standard method, by incinerating the diets at 550°C for 16 h in a muffle furnace. Energy content was determined by a ballistic bomb calorimeter. All of the above analysis followed standard AOAC (1980) procedure (Table2).

Table 1. The ingredients (each given as a percentage of the total on the basis of weight) and the proximate composition of the six test diets.

	Test diets					
	A1	A2	A3	CM	MW	AN
Ingredients						
Fish meal	50	50	50	-	-	-
Wheat meal	15	15	15	-	-	-
Soybean flour	20	20	20	10	-	-
Bread powder	-	-	-	90	-	-
Vitamin mineral premix	5	5	5	-	-	-
Cod-liver oil	10	-	-	-	-	-
Soybean oil	-	10	-	-	-	-
Coconut oil	-	-	10	-	-	-
Proximate composition (% dry basis)						
Crude protein	39.30	40.10	39.70	27.10	45.3	52.2
Fat (ether extract)	12.60	12.80	12.40	4.30	19.8	18.9
Crude fibre	0.33	0.41	0.55	0.58	-	-
Energy (cal/g)	52.70	55.40	53.10	35.10	-	-

Table 2. Total length, wet weight and SGR of guppy larvae fed with diets CM, AN, MW, A1, A2 and A3 and the unfed negative control (UC)[1].

	Diets						
	CM	MW	AN	A1	A2	A3	UC
Total length (mm) (Mean±SEM)							
5th day	$09.3^{a}\pm0.03$	$11.8^{b}\pm0.03$	$12.4^{c}\pm0.09$	$10.1^{d}\pm0.07$	$10.1^{d}\pm0.06$	$10.1^{d}\pm0.06$	$09.0^{e}\pm0.05$
10th day	$10.1^{a}\pm0.04$	$12.7^{b}\pm0.08$	$14.5^{c}\pm0.12$	$11.8^{d}\pm0.09$	$11.8^{d}\pm0.10$	$11.7^{d}\pm0.11$	$09.6^{e}\pm0.06$
15th day	$11.4^{a}\pm0.06$	$14.5^{b}\pm0.20$	$15.3^{c}\pm0.06$	$12.5^{d}\pm0.10$	$12.5^{d}\pm0.09$	$12.4^{d}\pm0.09$	$10.4^{e}\pm0.09$
20th day	$12.2^{a}\pm0.06$	$15.9^{b}\pm0.18$	$16.6^{c}\pm0.08$	$14.4^{d}\pm0.10$	$14.5^{d}\pm0.10$	$14.5^{d}\pm0.08$	-
Wet weight (mg) (Mean±SEM)							
20th day	$11.9^{a}\pm0.32$	$29.1^{b}\pm1.21$	$34.2^{c}\pm0.562$	$04.3^{d}\pm0.71$	$23.4^{d}\pm0.67$	$23.6^{d}\pm0.59$	-
SGR (% day^{-1})	6.2^{a}	10.6^{b}	11.6^{c}	8.5^{d}	8.4^{d}	8.5^{d}	-

[1] Within rows means with different superscripts are significantly different ($p<0.05$).

Feeding trials

Newly hatched guppy larvae were collected from the broodstock, maintained at the Department of Animal Science, University of Peradeniya, and reared under ambient conditions at a stocking rate of 20 larvae per 10 l aquarium and fed with the test diets once per day *ad libitum*. The diets were tested in triplicate and two batches of fish (in two tanks) served as unfed negative control following the standard procedure of Cahu *et al.* (1998). The feeding experiment was carried out for 20 days covering the entire larval period. Initial mean total length and mean wet weight were calculated from 15 randomly sampled larvae from the entire hatch. Thereafter on the 5th, 10th, 15th and 20th days all the living larvae were sampled for measurement of total length. As measurement of wet weights usually results in death of larvae, wet weights of the larvae were measured only on the 20th day.

Specific growth rates (SGR) were calculated as Tacon (1990):

$$SGR = \frac{[\ln(\textit{mean final wet weight}) - \ln(\textit{mean initial wet weight})]}{\textit{Number of days}} \times 100$$

Survival was measured using the following formula:

$$\textit{Survival}\,\% = \frac{\textit{Number of fish survived}}{\textit{Number of fish introduced}} \times 100$$

Maintenance of water quality

Tanks were cleaned by siphoning out the remaining feed, faeces and dead larvae, and one third of the water was exchanged daily. Mortality was recorded daily. Water quality parameters such as temperature, pH and dissolved Oxygen were recorded once in five days by using standard electronic probes. Ammonia content was measured by spectrophotometric method.

Statistical analysis

Data on total lengths, wet weights and SGR were analysed by the analysis of variance procedure to determine the differences among treatments. The means were compared using Duncan's test. The statistical model used was as follows:

$$Y_{ij} = \mu + Diet_i + e_{ij}$$

Where;

Y_{ij} = total length at 5th, 10th, 15th and 20th days; wet weight on 20th day; or SGR

μ = overall mean

$Diet_i$ = ith treatment effect (i: 1=CM, 2=MW, 3=AN, 4=A1; 5=A2; 6=A3 and 7=UC)

e_{ij} = residual effect

RESULTS AND DISCUSSION

Water temperature in the tanks (ranged from 25.9°C and 26.6°C) was within the range (20°C-28°C) recommended by Alabaster and Lloyd (1984). Variation of pH (between 7.2-7.7) was also within acceptable limits of 6.5 and 9.0 according to Boyd (1982). Dissolved oxygen levels measured (6.2-6.7 mg l^{-1}) were well above the lethal level of 3.0 mg l^{-1} (Boyd, 1982).

Many cyprinid larvae are quite tolerant to free ammonia. According to Boyd (1982), ammonia concentrations between 0.6 and 2.0 mg l^{-1} are toxic to many fish species. During the present experimental period in all the treatments, ammonia levels were found to be very low. When fish were fed with fish meal based formulated feeds (diets A1, A2 and A3), ammonia content was slightly higher. However, these values were within the optimum limits as the highest value recorded was 0.114 mg l^{-1}. Survival rates were more than 75% in all the treatments except in unfed control (Fig. 1). High survival rates of larvae, fed with the test diets indicate that even these diets have provided adequate energy.

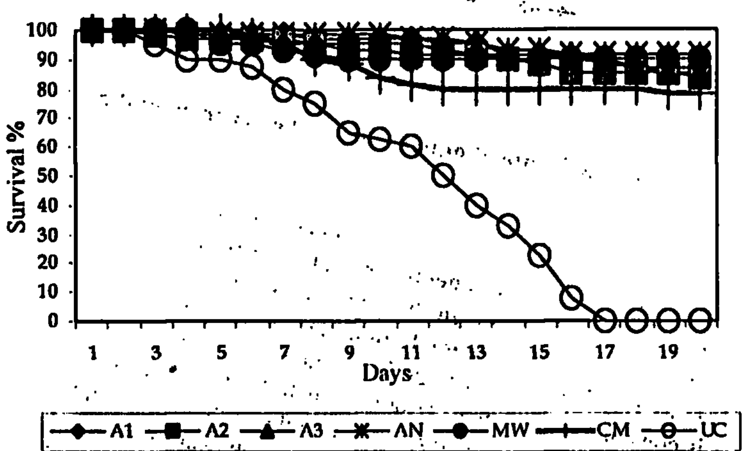

Fig. 1. Survival of guppy larvae fed with diets AN, MW, CM, A1, A2 and A3 and the unfed control (UC).

Total length of larvae showed a significant ($p<0.05$) variation among treatments (Table 2). However the differences among treatments A1, A2 and A3 were not significant ($p>0.05$). Similar results were obtained for final mean wet weights and SGR (Table 2; Fig. 2). The live feeds, AN and MW resulted in significantly better larval growth than all other diets ($p<0.05$). The results indicate that AN is the best feed tested in this study. Live feeds are more attractive to young fish larvae, which are mostly carnivorous hunters (Lavens *et al.*, 1995). Also live feeds make themselves more readily available to the fish by remaining in the water column by their peculiar movements for considerable time than sinking to the bottom, which usually is the case with formulated feeds.

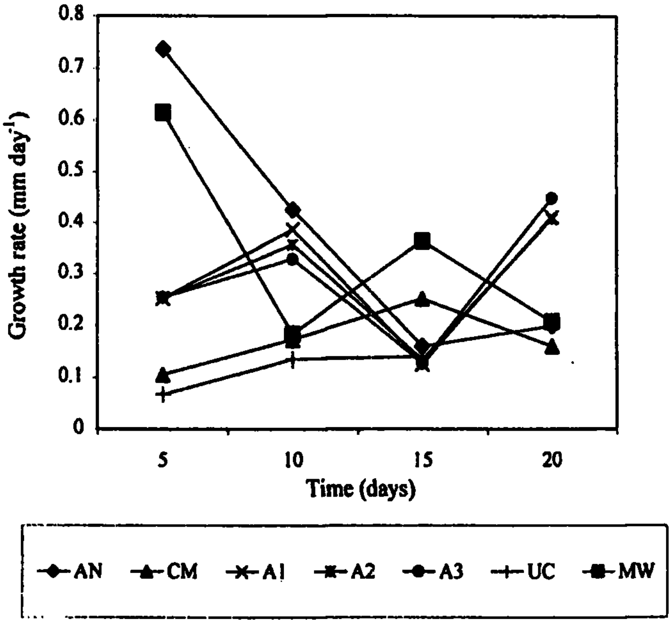

Fig. 2. Growth rate of guppy during the experimental period.

Apart from their high nutritional value (Browne *et al.*, 1991) live AN can actively swim up to 5 h in freshwater before sinking to the bottom and dying (Hoff and Snell, 1989). In this study MW, though they do not have the ability of active swimming, remained in suspension for a comparable time period as a result of weak water currents caused by aeration. This could be regarded as an important factor when feeding behaviour of guppy is considered. Guppies are predominantly surface feeders and also actively feed on diets in suspension, but they rarely feed on the bottom (Dussault and Kramer, 1981). Nutrient loss from live feed is much less or negligible when compared to formulated artificial diets (Goldblatt *et al.*, 1980; Watanabe *et al.*, 1983). All these factors could have been the reasons for the significantly ($p<0.05$) higher growth of larvae fed with live feeds, AN and MW than those fed with formulated feeds. AN has higher protein and fat content than all other diets. AN also contains essential fatty acids (EFA) (Browne *et al.*, 1991). Content and composition of EFA in MW has not been reported. However, free living and plant parasitic nematodes often contain large lipid reserves even up to 41% of the dry weight (Barrett *et al.*, 1971). Certain free living nematodes have the ability to synthesize EFA from their culture medium (Krusberg, 1972). The significantly better larval growth in MW group than CM group suggests the better nutritional quality of MW over CM. Thus increased feeding as well as high nutritional quality of MW could be the contributing factors for higher growth.

There was no significant ($p>0.05$) difference in growth of larvae fed with diets A1, A2 or A3, which differed mainly in their source of lipid content. Cod-liver oil contains high amounts of polyunsaturated fatty acids (PUFA), about 30% of total fatty acids dominated by linolenic (n-3) series, whereas soybean oil and coconut oil contains very low PUFAs, about 60 and 12% respectively of total fatty acids dominated mainly by linoleic (n-6) series

(Tacon, 1990). The PUFA play a central role in larval fish nutrition as these PUFA are essential for maintenance of the structure and integrity of cellular membranes. Therefore, the fast growing fish larvae will have a particular requirement of PUFA. The predominant PUFA in fishes and shellfishes belong to the linolenic (n-3) series (Cowey and Sargent, 1979).

However, comparatively higher levels of linoleic (n-6) series PUFA have been reported in freshwater fishes and, some warm freshwater fishes mainly require linoleic (n-6) series PUFA (Takeuchi *et al.*, 1983; Kanazawa, 1985). Furthermore some fish have the ability of chain elongation and further desaturation of fatty acids (Farkas *et al.*, 1980; Pozernick and Wiegand, 1997). The lack of significant difference ($p>0.05$) of guppy larvae among groups A1, A2 and A3 could be due to low n-3 PUFA requirement or high n-6 PUFA requirement or the ability of biosynthesis of PUFA from relatively more saturated and shorter chained fatty acid or the combination of these factors.

Growth rate of fish during this experiment exhibited an uneven pattern which varied remarkably between live and formulated feeds with time (Fig. 2). During the early stage (5th day), the fish fed with AN and MW showed higher growth rates. Achieving a high growth rate in young fish larvae is very important with respect to their survival potential and metabolism (Pedersen, 1997) as mortality due to predation tends to decrease sharply with increasing body size (Peterson and Wroblewski, 1984). Availability and nutritional quality of live feeds might have enabled the fish to grow rapidly during this early period. However, this high growth rate declined rapidly with time. Sustainability of high growth rates demands high energy levels from the feed, which may be the reason for the gradual decline in growth rate in the latter part of the period. On the other hand when fish larvae were fed with A1, A2, A3 and CM growth rate by the end of 5th day was lower than that in 10th day. The possible reason for this could be increasing feed intake during the course of the experimental period as the fish become more and more adapted to these feeds. The drop in growth rate in the 15th day may be due to allocation of energy to the synthesis of tissues, which would have increased the weight but not the length of fish.

The results of this study indicate that live feeds are better than formulated feeds and also AN is a better feed than MW for growth of guppy larvae. The results also indicate that cod-liver oil, which is relatively expensive, can be replaced with soybean or coconut oils in formulated diets for guppy larvae. Replacement of fishery products with terrestrial crop products for aquaculture would reduce the pressure on fisheries for the production of fish oils to be used in aquaculture.

Table 3 shows the approximate cost of the feed per kg basis. Microworm seems to be the cheapest among all the diets excluding CM. Although MW production is cheaper, large scale production of MW may be a tedious process. In such circumstances formulated feeds can be favored over MW. This should be decided on the merits of individual farms with respect to their size, availability of space, labour, sales demand, *etc*.

For small scale farmers formulated feeds may have an added advantage over MW due to their longer shelf life and easy handling. In situations where low cost ingredients are readily available formulated feeds can be competitive and cheaper than even MW. Considering such circumstances development of better formulated feeds should be encouraged and further studies must be conducted to investigate other possible ingredients that can be used to reduce the cost further and improve nutritive value of the formulated feeds.

Table 3. Approximate cost of the test diets.

Test diet	Cost (in Sri Lankan rupees)
	Per kg of feed
AN	6250.00
MW	36.00
CM	27.00
A1	77.00
A2	68.00
A3	67.50

CONCLUSIONS

It is evident from this study that AN is the best feed for guppy larvae in terms of nutritional quality. However, the feed prices indicate that the other feeds tested in the study can be economically superior alternatives to AN depending on the culture system. In cases where production of large quantities of MW or other live feeds is problematic, formulated feeds can be a better alternative. This study also proves that relatively expensive cod-liver oil can be effectively replaced with cheaper soybean or coconut oil in the formulated larval feeds without causing any significant reduction in growth and survival of guppy larvae.

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REFERENCES

Alabaster, J.S. and Lloyd, K. (1984). Water Quality Criteria for Freshwater Fish, Buttrerworths, London, UK.

AOAC. (1980). Official methods of analysis, 13th Edition, Association of Official Analytical Chemists, Washington, DC, USA.

Barrett, J., Ward, C.W. and Fairbairn, D. (1971). Lipid metabolism in the free living nematodes *Panagrellus redivivus* and *Tubatrix aceti*. Comp. Biochem. Physiol. 38B: 279-284.

Bengston, D.A., Léger, P. and Sorgeloos, P. (1991). Use of *Artemia* as a food source for aquaculture. pp. 255-285. *In*: Browne, R.A., Sorgeloos, P. and Trotman, C.N.A. (Eds). *Artemia* Biology, CRC Press Inc., Boca Raton, USA.

Browne, R.A., Sorgeloos. P. and Trotman, C.N.A. (1991). *Artemia* Biology. CRC Press Inc., Boca Raton, USA.

Boyd, C.E. (1982). Water Quality Management of Pond Fish Culture, Elsevier, Amsterdam, The Netherlands.

Cahu, C., Zambonino Infante, J., Escaffe, A-M., Bergot, P. and Kaushik, S. (1998). Preliminary results on the sea bass (*Dicentrarchus labrax*) larvae rearing with compound diet from first feeding. Comparison with carp (*Cyprinus carpio*) larvae. Aquaculture. 169: 1-7.

Cowey, C.B. and Sargent, J.R. (1979). Fish nutrition. pp. 1-69. *In*: Hoar, W.S. and Randall, D.J. (Eds). Fish Physiology, Vol. III, Academic Press, New York and London, UK.

Dussault, G.V. and Kramer, D.L. (1981). Food and feeding behaviour of guppy, *Poecilia reticulata* (Pisces - Poeciliidae). Can. J. Zool. 59: 684-701.

Edirisinghe, U. (1999). Ornamental fisheries as a livelihood for coastal regions. pp. 63-70. *In*: Gunasena, H.P.M., Kotagama, H.B. and Wickramasuriya, H.V.A. (Eds). Coastal Resource Management in Sri Lanka, Workshop Proceed. August 6, 1999, University of Peradeniya, Sri Lanka.

Farkas, T., Csengeri, I., Majors, F. and Oláh, J. (1980). Meatbolism in fatty acids in fish III. Combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, *Cyprinus carpio* Linneaus 1758. Aquaculture. 20: 29-40.

Goldblatt, M.J., Conklin, D.E. and Brown, D. (1980). Nutrient leaching from coated crustacean rations. Aquaculture. 19: 383-388.

Hirimuthugoda, N.Y., Edirisinghe, U. and Gunawardena, A. (1999). Determination of suitability of egg deposition materials and development of larval feeds for higher growth and survival of common carp (*Cyprinus carpio* L.). Trop. Agric. Res. 11: 229-238.

Hoff, F.H. and Snell, T.W. (1989). Plankton Culture Manual, Florida Aqua Farms Inc., Pisces Publishing Group, Devon, USA.

Kanazawa, A. (1985). Essential fatty acid and lipid requirement of fish. pp. 281-298. *In*: Cowey, C.B., Mackie, A.M. and Bell, J.G. (Eds). Nutrition and Feeding in Fish, Academic Press, London and New York, UK.

Krusberg, L.R. (1972). Fatty acid composition of *Tubatrix aceti* and its culture medium. Comp. Biochem. Physiol. 41B: 89-98.

Lavens, P., Sorgeloos, P., Dhert, P. and Deveresse, B. (1995). Larval foods. pp. 373-397. *In*: Bromage, N.R. and Roberts, R.J.(Eds). Broodstock Management and Egg and Larval Quality, Blackwell Science, Oxford, UK.

Mee, J.K.L. (1993). An Overview of the Ornamental Aquatics Sector in Sri Lanka. AgEnt 08-93, AgEnt Consultant Report No. 01, Colombo, Sri Lanka.

NARA. (1999). Sri Lankan Fisheries Yearbook 1999. National Aquatic Resources Research and Development Agency (NARA), Colombo, Sri Lanka.

Pedersen, B.H. (1997). The cost of growth in young fish larvae, a review of new hypothesis. Aquaculture. 155: 259-269.

Peterson, I. and Wroblewski, J.S. (1984). Mortality rate of fishes in the pelagic ecosystem. Can. J. Fish. Aquat. Sci. 41: 1117-1120.

Pozernick, M. and Wiegand, M.D. (1997). Use of canola oil in the feed of larval and juvenile goldfish, *Carassius auratus* (L.). Aquaculture Res 28: 75-83.

Tacon, A.G.J. (1990). Standard Methods for the Nutrition and Feeding of Farmed Fish and Shrimp, Argent Laboratories Press, Redmond, Washington, USA.

Takeuchi, T., Satoh, S. and Watanabe, T. (1983). Requirement of *Tilapia nilotica* for essential fatty acids. Bull. Jap. Soc. Sci. Fish. 49: 1127-1134.

Watanabe, T., Kitajima, C. and Fujita, S. (1983). Nutritional value of live organisms used in Japan for mass propagation of fish: a review. Aquaculture. 34: 115-143.