 DOI: 10.11598/btb.2019.26.3.1103

Spirulina platensis DIET FOR MILKFISH, *Chanos chanos*, LARVAE**

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Received 20 July 2018 / Accepted 06 December 2018

ABSTRACT

In aquaculture, *Spirulina platensis* is used as feed supplement in which contains amino acid phenylalanine. This study was conducted to evaluate the differences in the effect of spirulina-based microcapsules and commercial diets on the absolute, daily and specific growth and survival rates of milkfish larvae. The larvae were fed with *Spirulina platensis* as a core diet in microcapsules with different matrix (walls). The first capsule wall was gelatin and fish oil, while the second capsule wall was gelatin, fish oil and whole egg. The control group was fed with the commercial diet. A total of 1200 larvae were used in this experiment using the recirculation systems. The experiment was conducted in 42 days of culture. Larvae were fed three times a day and the feed was increased regularly as the size of the larvae increased. The results showed that the effects of both sprirulina-based microcapsules diets on the absolute growth rate (AG), specific growth rate (SGR) and average daily growth rate (ADGR) of *Chanos chanos* larvae were the same as on those larvae which were fed with the commercial diet. The survival rates were at 80.6±11.17% for those fed with *Spirulina platensis* with gelatin and fish oil wall; 84.6±8.44% for those fed with *Spirulina platensis* with gelatin, fish oil and whole egg wall, and; 83.8±16.50% for those fed with the commercial diet. This study showed that Spirulina-based microcapsules had the same effect as the commercial feed on the growth of milkfish larvae indicating that this diet could replace the commercial diet.

Keywords: *Chanos chanos*, microcapsule wall, *Spirulina*

INTRODUCTION

Milkfish *Chanos chanos* Forsskal, Orange-spotted grouper *Epinephelus coioides*, hard-lipped barb *Osteochilus hasselti* and giant gouramy *Osphronemus goramy* Lacepede are particularly favored in Indonesia, especially in Java because they are easy to breed and their flesh is favored (Yuwono & Sukardi 2009; Prayogo *et al.* 2016a, 2016b; Sukardi *et al.* 2018). In the brackishwater ecosystem, the fishes, crustaceans and other aquatic organisms larvae consume a variety of micro- and macro-algae which have good nutritional composition such as protein, lipids, fatty acids, and vitamins. These components promote growth and are immune enchancers (van Dam *et al.* 2002; Ju *et al.* 2008; Ju *et al.* 2009; Kuhn *et al.* 2010; Supamattaya *et al.* 2005; Van Der Meeren *et al.* 2007; Sudaryono *et al.* 2018). After yolk sac absorption, the milkfish larvae, like other fish species, need sufficient and continuous source of live food like rotifer, *Brachionus plicatilis* and *Artemia.* Hence, hatcheries use green-water containing phyto- and zooplankton (Tamaru *et al.* 1994; van Dam *et al.* 2002; Soomro *et al.* 2015). Formulated microcapsule diets using single cell protein-based ingredients present an alternate approach to improve the delivery of essential nutrients to the larvae. Microencapsulation technique allows the manufacture of stable small capsules that may prevent nutrient leaching, is easy to handle, and is environmentally friendly (Aragão *et al.*

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**This paper was presented at the 2nd Scientific Communication in Fisheries and Marine Sciences (SCiFiMaS 2018), 07-09 May 2018, Purwokerto, Central Java, Indonesia

2014; Dubey *et al.* 2009; Umer *et al.* 2011; Wilson & Shah 2007). Microencapsulated diets appear to be a good option to overcome some limitations in fish diets. Microcapsule diet substitution for live prey is, therefore, critical in lowering the production cost and ensuring sustainable supply of high quality fish feed. A number of different formulas of microencapsulated diets have been developed and experienced extensively for several species of crustaceans, including the *Penaeus japonicus* Bate (Xie *et al.* 2010), bivalve lions-paw scallop, *Nodipecten subnodosus* (Saucedo *et al.* 2013), and fish, larval Halibut, *Hippoglossus hippoglosus*. Hence, this research aimed to evaluate the different effects of spirulina-based microcapsules and commercial feed on the absolute, daily and specific growth and survival rates of milkfish larvae and of the different microcapsule wall types on the fish growth.

MATERIALS AND METHODS

Study Design

A recirculating tank system was applied in which every tank was aerated with air stones. Three groups of experiments were carried out wherein each group consisted of three cylinder tanks of 50 L capacity, containing 100 fish or fingerlings of 1-2 cm long, having weight of 0.11-0.21 g in each tank (equivalent to the density of one fish in a 2 L water volume) maintained at 27-29°C, for the feeding trials. Each treatment group had three replications. The microencapsulated diets were formulated using two different wall materials. The first spirulina capsule wall consisted of gelatin and fish oil (Treatment 1). The 2nd wall consisted of eggs, gelatin and fish oil (Treatment 2). Fish oil was used as an attractant flavor. The control group (Treatment 3) was the commercial feed. The alga species, *Spirulina platensis*, was cultured as described previously by Sukardi *et al.* (2014). The algal species included in the microencapsulated diets were harvested when the algae reached the stationary phase at a density of 73,442 x 10^4 cell.mL^{-1} *Spirulina platensis*. The capsule particles were produced by a modified method of the thermal cross-linking technique, as described by Sukardi *et al.* (2014, 2018). Microcapsules were prepared by mixing one part of wall (matrix) with one part of inclusion. The ratios are described in Table 1 and 2.

Table 1 Composition of microencapsulated diet for feeding experiment (Treatment 1)

No.	Diet components	% composition by weight
1.	Matrix : (60%/w)	
	Gelatin	42
	Fish oil	18
2.	Inclusion (40%/w)	
	Spirulina platensis	32
	Vitamin mix	4
	Lysine	4

Table 2 Composition of microencapsulated diet for feeding experiment (Treatment 2)

No.	Diet components	% composition by weight
1.	Matrix : (60%/w)	
	Eggs	42
	Gelatin	12
	Fish oil	6
2.	Inclusion (40%/w)	
	Spirulina platensis	32
	Vitamin mix	4
	Lysine	4

The first and second capsules had a final composition of 57.4% and 47.5% crude protein, respectively, whilst the control feed was 41%. The fish larvae were cultured with a series of microencapsulated and commercial diets in brackishwater (15-25‰). The diets were fed to fish larvae three times daily during the 42-day experiment. During the first several days, feeding rates increased periodically as the larvae increased in size.

Growth Parameters

The absolute growth is the weight gain (g), AG(g)=Wt–Wi, where Wt is the final weight (g) and Wi is the initial weight (g). Average daily growth rate, ADGR=[{Wt–Wi}/T], where Wt is the final weight (g), Wi is the weight of the fish at time 0 and T is the culture period in days of experiment. Specific Growth Rate, SGR (%/d)=100[(lnWt–lnWi)/T], where Wt is the final weight (g), Wi is the weight of fish at time 0, and T is the culture period in days of experiment. The survival rate (%)=(Total number of fish that survived)/(Total number of stocked fish)x100.

Statistical Analysis

The arch-sine square root transformation was applied to all percentage data prior to analysis. A one-way analysis of variance (ANOVA) was used to determine whether significant differences existed among the treatments. Then, Tukey's procedure was used to determine significant differences among the treatments. Statistical analysis was done using SPSS for Windows (V.24).

RESULTS AND DISCUSSION

The capsules were measured microscopically and the diameters ranged from about 100.98 to 187.94 μm and the average was 145.93 ± 20.95 μm. These *Spirulina* microcapsules had adequate shape and size, and were stable in the brackish-water (Fig. 1). The length of larvae was about 2-2.5 cm.

After 42 days of treatment, *Chanos chanos* obtained a weight gain of 0.1182 ± 0.055 g; 0.1902 ± 0.043 g and 0.2230 ± 0.086 g, for Treatment 1, Treatment 2 and control group, respectively (Fig. 2). No significant differences ($P>0.05$) exist in the absolute growth of *Chanos chanos* larvae which were fed with *Spirulina* microcapsules and of those fed with the commercial diet. This implies that the nutritional components of *Spirulina*-based microcapsules fulfilled the growth requirements of *Chanos chanos* larvae, the same as that of the commercial diet. This study showed that gelatin-walled and mixture-walled (gelatin and egg) capsules had the same effect on fish growth. In *Macrobrachium rosenbergii* (de Man) larvae, the acceptance of the microencapsulated diet by the larvae was more than 70% (Anas *et al.* 2008). The other study revealed that protein-walled capsules are better than lipid-walled capsules for larval performance (Langdon 2003). Microencapsulated diets have been found to support larval growth when fed in combination with live diet Tubifex worm (Sukardi *et al.* 2018).

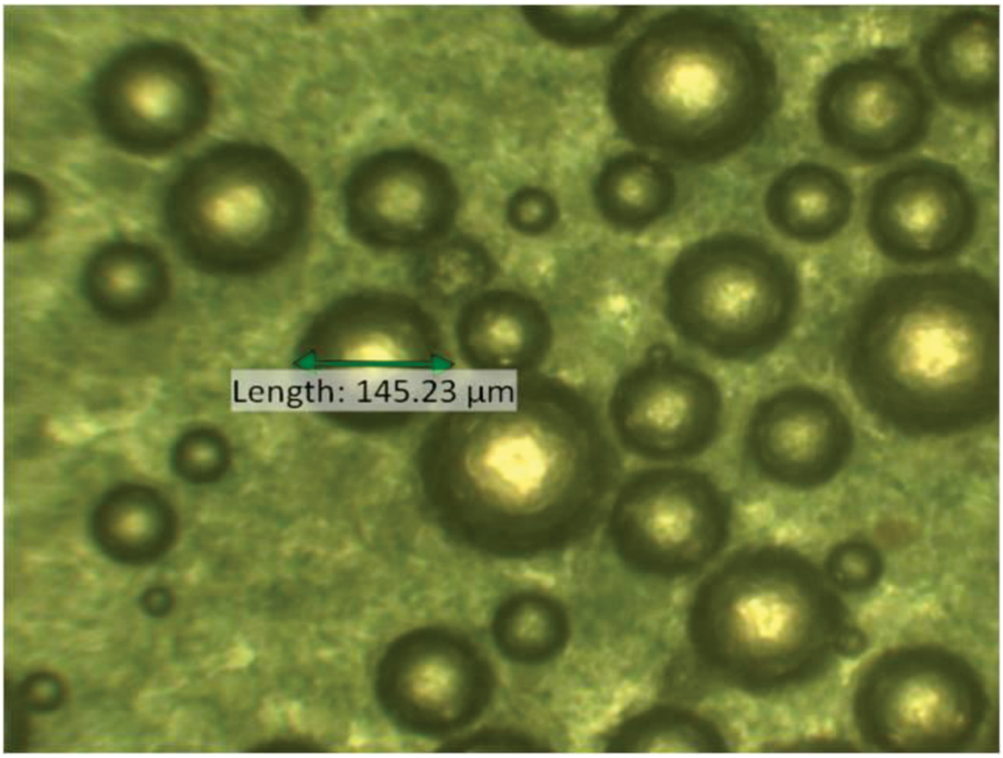

Figure 1 A microphotograph showing the *Spirulina platensis*-based microcapsules using the light microscope Boeco 10 x 10 magnification

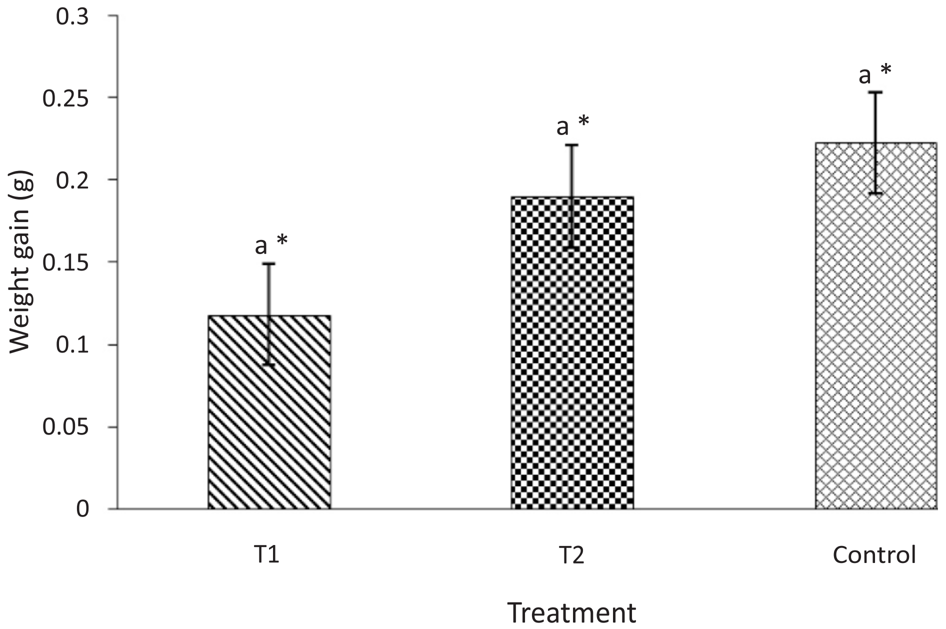

Figure 2 Absolute growth of *Chanos chanos* larvae reared for 42 days of culture (bars with the same superscript do not significantly differ (P>0.05))

The specific growth rates (SGR) of *Chanos chanos* fed with *Spirulina* microcapsule 1, 2 and the control were 1.39 ± 1.16%/d, 2.16 ± 0.95%/d and 2.00 ± 1.55%/d, respectively (Fig. 3). The SGR of *Chanos chanos* fed with both microcapsule diets and those fed with the commercial diet did not significantly differ (P>0.05) which means that the nutritional component inside the microcapsules had same effect as that of commercial diet.

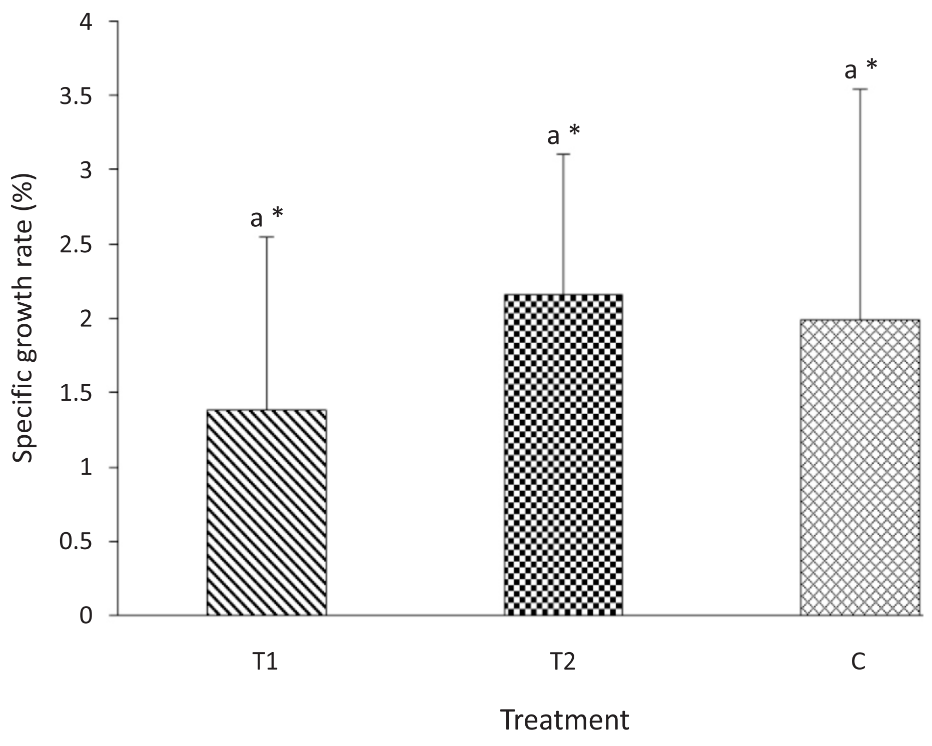

Figure 3 The specific growth rate (SGR) performance of *Chanos-chanos* after 42 days of culture

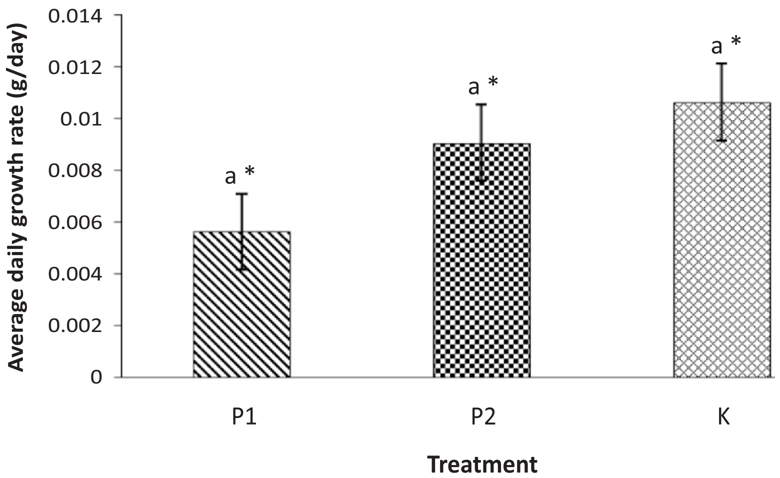

Figure 4 Average daily growth rate (ADGR) of milkfish, *Chanos chanos* larvae after 42 days of culture (bars with the same superscript do not significantly differ (P>0.05))

The ADGR of *Chanos chanos* larvae which fed on *Spirulina*-based microcapsules (Treatments 1 and 2) and the control group (fed with commercial diet) were at 0.0028 ± 0.001 g/day; 0.0050 ± 0.001 g/day and 0.0053 ± 0.003 g/day, respectively (Fig. 4). The effect of both *Sprirulina*-based microcapsule diets on the absolute growth rate (AG), specific growth rate (SGR) and average daily growth rate (ADGR) of *Chanos chanos* larvae is similar with those fed with the commercial diet. This implies that the nutritional component inside the microcapsule matched the requirements for larval growth. Other studies showed that good larval growth was only achieved with micro-diets if feeding with live prey took place. Live feed enrichment could improve the utilization of micro-diets. Larval red sea bream, *Pagrus major* and Japanese flounder, *Paralichthys olivaceus* fed with micro-diet together with live feed were able to maintain growth and survival (Kanazawa *et al.* 1989). The micro-diet was prepared using an internal gelation method to partially substituted the traditional live food (*Artemia*) for larval Atlantic halibut, *Hippoglossus hippoglossus* L. (Murray *et al.* 2010). In the rearing of marine fish larvae, gilthead sea bream, *Sparus aurata* L., the live food was substituted with microencapsulated diets, however, only limited growth was achieved (Langdon 2003; Yúfera *et al.* 1999). For giant-gouramy, *Osphronemus goramy*, a micro-diet together with Tubifex worm was only effective if introduced at 22 days after hatching (Sukardi *et al.* 2018). A kappa-carrageenan-based micro-diet was also suitable for *Penaeus japonicus* larvae (Koshio *et al.* 1989).

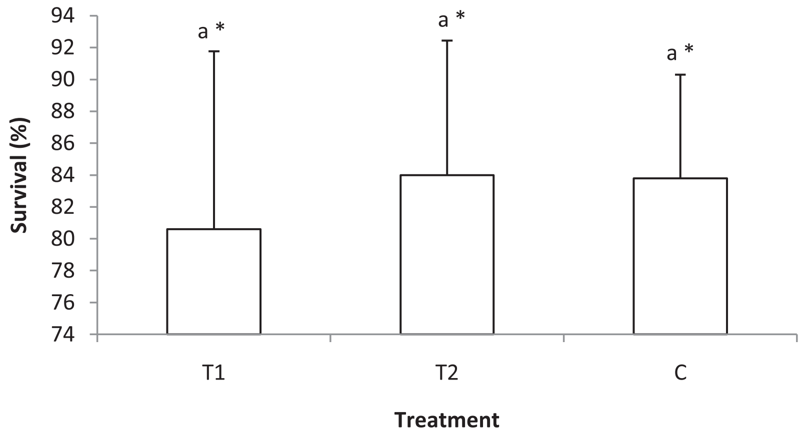

Figure 5 Survival rates of *Chanos chanos* larvae reared after 42 days (bars with the same superscript do not significantly differ (P>0.05))

The survival of *Chanos chanos* larvae was 80.6 ± 11.17%, 84.6 ± 8.44%, 83.8 ± 16.50% (Fig. 4). The milkfish larvae achieved more than 80% survival. This was higher compared to the survival rate (32.7%) of larvae fed with phytoplankton, rotifers and brine shrimp nauplii (Eda *et al.* 1990). However, this study's results were lower compared to *Chanos chanos* larvae (94-97%) fed with diets containing white fish meal and zein supplemented with amino acids (Borlongan & Benitez 1990).

CONCLUSION

Microencapsulated diet manifested a good/viable prospect as larval diet for milkfish, *Chanos chanos*. Although changing the physical properties, the chemical composition and the formulation of microcapsules, such as particle size, amino acid composition, have improved the quality and health of milkfish larvae, growth is still limited to fish responses as in other micro-diets.

ACKNOWLEDGEMENTS

This study was supported by a grant from The Ministry of Research, Technology, and Higher Education, Republic of Indonesia, under "Hibah Bersaing-Grant" and Universitas Jenderal Soedirman, Indonesia.

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