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ACCEPTED MANUSCRIPT

18 **A DIETARY MICROCAPSULE BASED ON SINGLE CELL PROTEIN (*Spirulina platensis*)**  
19 **FOR MILK FISH, CHANOS-CHANOS, LARVAE\*\***

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31 Running title: Single cell protein diet for milk fish larvae

32  
33 **ABSTRACT**

34 The purpose of this study was to evaluate differences between spirulina-based  
35 microcapsules and commercial diets on absolute, daily and specific growth and survival rates of  
36 milkfish larvae. *Spirulina platensis* was as a core diet in microcapsules with different matrix  
37 (walls). The first capsule wall was gelatin and fish oil, while the second capsule wall was gelatin,  
38 fish oil and whole egg. The control group was commercial diet. A total of 1200 larvae were used in  
39 this experiments using recirculation systems. Larvae are fed three times a day and increased  
40 regularly when the size of the larvae increases. The results showed that the effect of both spirulina-  
41 based microcapsule diets on the absolute growth rate (AG), specific growth rate (SGR) and average  
42 daily growth rate (ADGR) of Chanos-chanos larvae fed spirulina-based microcapsule same as them  
43 which to be fed using commercial diet. The survival rate was as 80.6±11.171%; 84.6±8.443%;  
44 83.8±16.496%, respectively. This study showed that Spirulina-based microcapsules had the same  
45 effect as commercial feed on the growth of milkfish larvae which means that this diet could replace  
46 commercial diet.

47  
48 **Keywords:** microcapsule wall, Spirulina, Chanos chanos

49  
50 **INTRODUCTION**

51 Milk fish (*Chanos-chanos* Forskal), Orange-spotted grouper (*Epinephelus coioides*), hard-  
52 lipped barb (*Osteochilus hasselti*) and giant gouramy (*Osphronemus gourami* Lacepede) are  
53 particularly favored in Indonesia especially in Java because they are easy to breed and their flesh is  
54 favored (Yuwono and Sukardi, 2009; Prayogo et al. 2016<sup>a</sup>,2016<sup>b</sup> and Sukardi, et al., 2018). In the  
55 brakishwater, fish, crustaceans and other aquatic organisms larvae consume a variety of micro and  
56 macro-algae which has good nutritional composition such as protein, lipids, fatty acids, and  
57 vitamins. These components are essential to promote growth and immune enhancers (van Dam, et  
58 al. 2002; Ju et al., 2009; Kuhn et al., 2010; Supamattaya et al., 2005; Van Der Meeren et al., 2007,  
59 Sudaryono et al., 2018). In the brakishwater, fish, crustaceans and other aquatic organisms larvae  
60 consume a variety of micro-algae which has good nutritional composition such as protein, lipids,

61 fatty acids, and vitamins. These components are essential to promote growth and immune  
62 enhancers (van Dam, et al. 2002; Ju et al.,2009; Kuhn et al.2010; Supamattaya, et al. 2005; Van  
63 Der Meeren et al., 2007).

64 Milk fish larvae, like other fish species, after yolk sac absorption, need sufficient and  
65 continuous source of live food such as rotifer, *Brachionus plicatilis* and *Artemia*, therefore in the  
66 hatchery applied green-water in which consisted of phyto-and zooplankton (Tamaru et al., 1994;  
67 van Dam, 2002; Soomro, et al. 2015). Formulated microcapsul diets using single cell protein-base  
68 ingredients represent an alternative approach to improve the delivery of essential nutrients to the  
69 larvae. Microencapsulation is a technique which allows the manufacture of stable small capsules  
70 that may prevent nutrient leaching, easy to handle, and environmentally friendly (Aragao, et  
71 al.,2014; Dubay et al., 2009; Umer, et al., 2010, Wilson and Shah, 2007). Microencapsulated diets  
72 appear to be a good option to over come these limitations. Microcapsule diet substitution for live  
73 prey is therefore important for lowering production cost and ensuring sustainable supply of high  
74 quality fish seed. A number of different formula of microencapsulated diets have been developed  
75 and experienced extensively for several species of crustaceans include *Penaeus japonicus* Bate (Xie,  
76 et al.,2010), bivalve lions-paw scallop, *Nodipecten subnodosus* (Saucedo, et al.,2013), and fish,  
77 larval Halibut (*Hippoglossus hippoglossus*). The purpose of this research was to evaluate the  
78 difference of spirulina-based microcapsules and commercial feed to absolute, daily and specific  
79 growth and survival rate of milkfish larvae and difference of microcapsule wall types on the fish  
80 growth.

## 81 82 **MATERIALS AND METHODS**

### 83 **Writing the Materials and Methods**

84 A recirculating system were applied in which every tanks aerated with air stones. Three  
85 group experiments were carried out wherein each group consisted of three cylinder tanks (50 L)  
86 contained 100 fish with size of 1-2 cm and weight of 0,11-0,21g ( equivalent to a fish density of 2 L  
87 water volume) maintained at 27-29<sup>0</sup>C, for feeding trials. Each of the group was conducted using  
88 three tanks randomly. Microencapsulated diets were designed using two different wall materials, the  
89 first spirulina capsule (treatment 1) was designed use wall consisted of gelatin and fish oil, whereas  
90 the second one (treatment 2) was eggs, gelatin and fish oil. **Fish oil used as an attractant flavor.**  
91 The control group (treatment 3) was commercial feed. The alga species, *Spirulina platensis*, was  
92 cultured as described previously (Sukardi, et al. 2014). The algal species as inclusion materials of  
93 microencapsulated diets were harvested when reach stationary phases at a density of **73442 x 10<sup>4</sup>**  
94 **cell<sup>mL</sup>** *Spirulina platensis*. Capsule particles produced by a modification method of the thermal

95 cross-linking technique, as described Sukardi, et al. (2014, 2018). Microcapsules were prepared by  
96 mixing one part of wall (matrix) with one part of inclusion and the ratio was described as follows.

97

98

99 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

100

101 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

102

103 Fish larvae were cultured with a series of microencapsulated and commercial diets in brackish  
104 water (15-25 ppt). The diets were fed to fish larvae three times daily for 42 days. During the first  
105 several days, feeding rates were based on observation of feeding behavior of fish and increased  
106 periodically as the larvae increased in size.

107

### 108 **Growth parameter**

109 Absolute Growth = weight gain (g),  $AG(g) = W_t - W_i$ , where  $W_t$  is final weight (g),  $W_i$  is  
110 initial weight (g). Average daily growth rate= $ADGR = \frac{W_t - W_i}{T}$ , where  $W_t$  is final weight (g),  
111  $W_i$  is the weight of fish at time 0 and T is a culture periode in days of experiment. Specific Growth  
112 Rate= $SGR (\%/d) = 100 \frac{(\ln W_t - \ln W_i)}{T}$ , where  $W_t$  is final weight (g),  $W_i$  is the weight of fish at  
113 time 0, T is a culture periode in days of experiment. Survival( $\%$ )= $(\frac{\text{Total number of fish survived}}{\text{Total number of fish stocked}}) \times 100$ .

114

### 115 **Statistical analysis**

116

117 The arch-sine square root transformation was applied to all percentage data prior to analysis.  
118 A one way analysis of variance (ANOVA) was used to determine whether significant differences  
119 existed among treatments. Then, Tukey's procedure used when significant difference found  
120 amongst the treatments. Statistical analysis fulfilled using SPSS for Window (V.24).

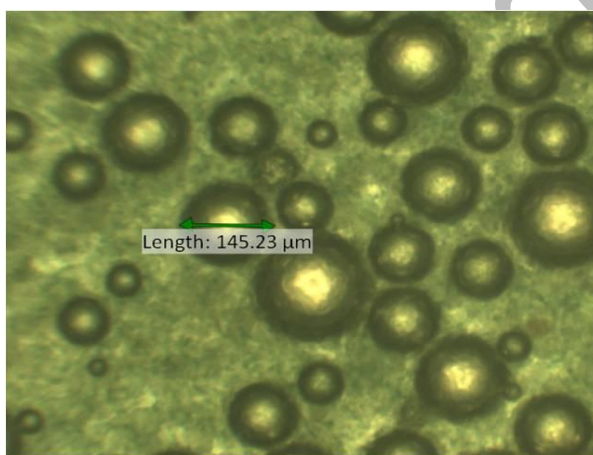
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## RESULTS AND DISCUSSION

123 The capsules were measured microscopically and the diameters ranged from about 100,98 -  
124 187,94 $\mu\text{m}$  and the average was 145,93 $\pm$  20.95 $\mu\text{m}$ . Spirulina microcapsules were adequate shape and  
125 size, stability in the brackish, as well (Fig.1). The length of larvae was about 2-2.5cm. The first  
126 capsule and the second had a final composition of 57.4% and 47.5% crude protein, respectively,  
127 whilst the control feed was 41%.

128

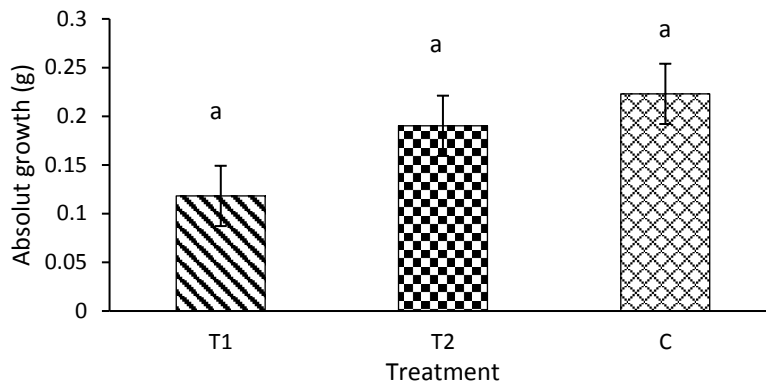


129

130 Figure 1 A Microphotograph showing the spirulina microcapsule (light microscope Boeco 10 x 10)

131

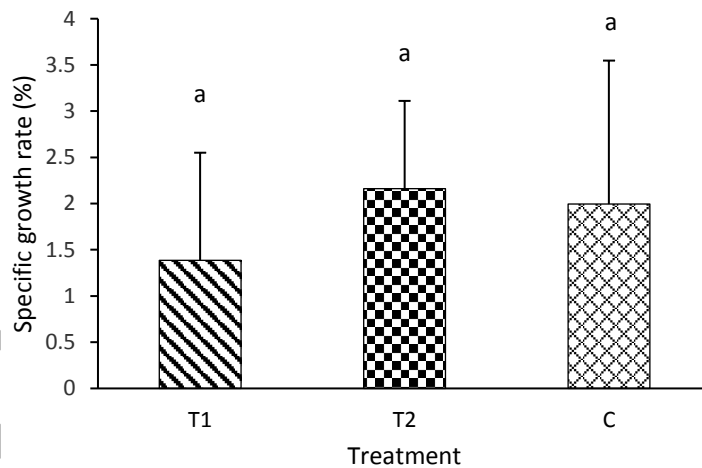
132 The absolute growth of *Chanos chanos* for period of 42 days showed in Fig.2. It was  
133 observed that upon the the harvest the fish in the treatment 1, treatment 2 and control group reached  
134 a weight of 0.1182  $\pm$  0.055 g; 0.1902  $\pm$  0.043 g and 0.2230  $\pm$  0.086 g, respectively. There was not  
135 significantly different ( $P > 0.05$ ) in the absolute growth of *Chanos chanos* larvae which were fed  
136 microcapsule based on *Spirulina platensis*. It indicate that the nutritional components of spirulina-  
137 based microcapsule fulfilled requirements for growth of *Chanos-chanos* larvae same as the  
138 commercial diet.



139

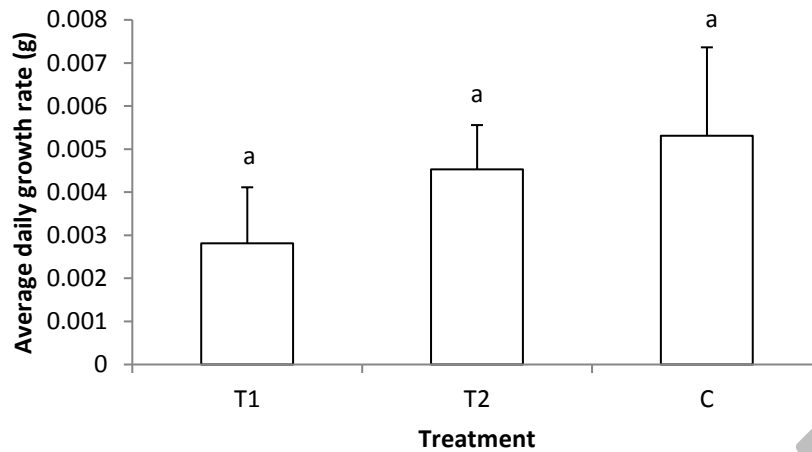
140 Figure 2 Absolute growth of *Chanos-chanos* larvae reared during 42 days of culture. Bars  
 141 represented by same superscript letters indicate not significantly different values ( $P >$   
 142 0.05).  
 143

144 It can be seen (Fig. 3) that specific growth rate of *Chanos-chanos* fed spirulina microcapsule  
 145 1, 2 and the control was  $1,39 \pm 1,16\%/d$ ;  $2,16 \pm 0,95\%/d$ ; and  $2,00 \pm 1,55\%/d$ , respectively. The  
 146 SGR of *Chanos chanos* fed both spirulina microcapsule diets and the control were not significantly  
 147 different ( $P > 0.05$ ).  
 148



149

150 Figure 3 The specific growth rate (SGR) performance of *Chanos chanos* during 42 days of culture  
 151

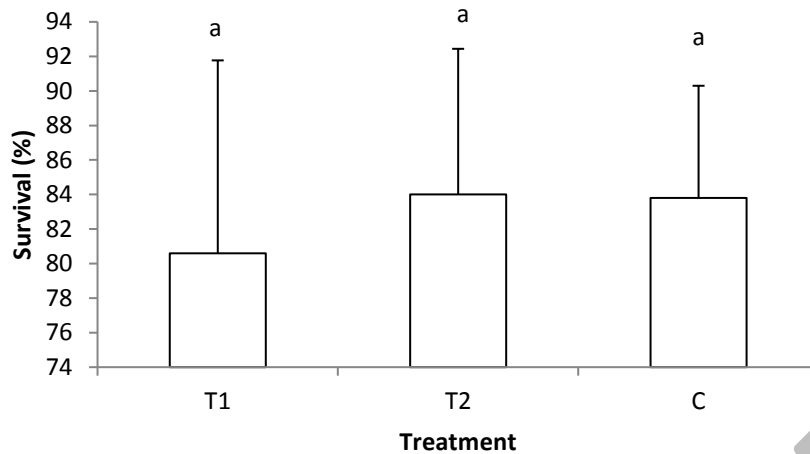


152

153 Figure 4 Average daily growth rate (ADGR) performance of milk-fish, *Chanos chanos* larvae  
 154 during 42 days of culture. Bars represented by same superscript letters indicate not  
 155 significantly different values ( $P > 0.05$ ).  
 156

157 ADGR of *Chanos-chanos* larvae which fed spirulina-based microcapsule on the treatment 1,  
 158 2 and control group was  $0,0028 \pm 0,001$  g/day;  $0,0050 \pm 0,001$  g/day; dan  $0,0053 \pm 0,003$  g/day,  
 159 respectively (Fig.4). The effect of both spirulina-based microcapsule diets on the absolute growth  
 160 rate (AG), specific growth rate (SGR) and average daily growth rate (ADGR) of *Chanos-chanos*  
 161 larvae same as the commercial diet. It means that nutritional component inside the microcapsule  
 162 matched the requirements of the larvae for their growth. In some studies showed that good larval  
 163 growth were only achieved with micro-diets if feeding with live prey takes place. Live feed  
 164 enrichment could improve the utilization of micro-diets. Larval red sea bream, *Pagrus major*, and  
 165 Japanese flounder, *Paralichthys olivaceus* fed micro-diet together with live feed could keep up the  
 166 growth and survival (Kanazawa, et al. 1989). Microdiet prepared using an internal gelation method  
 167 was used to partially substitute the traditional live food (*Artemia*) for larval Atlantic halibut,  
 168 *Hippoglossus hippoglossus* L. Microcapsule can be used to partially substitute the live food,  
 169 *Artemia*, for Atlantic halibut, *Hippoglossus hippoglossus* L. larvae (Murray, et al. 2010). In the  
 170 rearing marine fish larvae, gilthead sea bream, *Sparus aurata* L., live food could be substitute with  
 171 microencapsulated diets, however, only limited growth was achieved (Langdon 2003; Yúfera et al.  
 172 1999). For Giant-gouramy *Osphronemus gouramy*, a micro-diet together with Tubifex worm was  
 173 only effective if introduced 22 days post hatching (Sukardi et al, 2018). A kappa-carrageenan-based  
 174 micro-diet was also suitable for *Penaeus japonicus* larvae (Koshio et al., 1989).

175



176

177 Figure 5 Survival rates of Chanos-chanos larvae reared during 42 days of culture. Bars represented  
 178 by same superscript letters indicate not significantly different values ( $P > 0.05$ ).  
 179

180 It can be seen in Fig. 4, the survival of Chanos chanos larvae was  $80,6 \pm 11,17$  %;  
 181  $84,6 \pm 8,44$ %;  $83,8 \pm 16,50$  %. More than 80% survival of milk fish larvae was achieved in this  
 182 experiment, which was higher as compared to the survival (32.7%) larvae fed phytoplankton,  
 183 rotifers and brine shrimp nauplii (Eda, et al.1990). However, it was lower compared to Chanos-  
 184 chanos larvae (94-97%) fed diets contained white fish meal and zein supplemented with amino  
 185 acids (Borlongan and Benitez, 1990).

186

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189

### CONCLUSION

190 Microencapsulated diet showed prospect as a larval diet in milk-fish, Chanos-chanos,  
 191 although not entirely successful. Growth is still limited to fish as in other micro-diets. Ever-  
 192 changing the physical properties and chemical composition and the formulation of micro-capsules,  
 193 such as particle size, amino acid composition, will improve the quality and health of milk fish  
 194 larvae.

195

196

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