EFFECT OF MICROALGAE ON GROWTH AND FATTY ACID PROFILES OF HARPACTICOID COPEPOD, *Tisbe holothuriae*

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ABSTRACT

Growth of marine copepods is influenced by feed. The purposes of this trial were to observe both growth and fatty acid compositions of harpacticoid copepod nauplii, *Tisbe holothuriae* by feeding with several microalgal species in laboratory: (A) *Isochrysis tahiti*; (B) *Nannochloropsis oculata*; (C) *Rhodomonas* sp., and (D) *Tetraselmis chuii*. The trial was carried out for 35 days with randomized complete design and triplicates in each treatment. The results showed that final copepod nauplii densities were not significantly different ($P>0.05$) in all treatments. However, lipid content of copepod nauplii fed with *T. chuii* was significantly higher ($P<0.05$) compared to that of other treatments while fatty acid profiles of EPA, DHA and DHA/EPA ratios showed both insignificant and significant differences among treatments.

KEYWORDS: fatty acids, harpacticoid copepods, microalgae, *Tisbe holothuriae*

INTRODUCTION

Copepods are one of the most abundant organisms and its biomass makes up the greatest portion of marine zooplankton population (Hickman, 1993). With over 11,500 species, they are more numerous than insects on land, even though insects are far more diverse (Humes, 1994). From a nutritional standpoint, copepod nauplii are often considered nutritionally better than rotifers (*Brachionus* sp.), brine shrimp (*Artemia* sp.) (Stottrup *et al*., 1998), and oyster trochophores (Lim, 1991; Doi & Singhagraiwan, 1993). One reason why copepod nauplii might be nutritionally better than other live food sources is that they have high content of HUFA (highly unsaturated fatty acids), PUFA (poly unsaturated fatty acids), and other fatty acids needed to meet the nutritional requirements of a fish's early ontogeny (Witt *et al*., 1984). In fish hatcheries, copepod nauplii are preferred as the first live food for many fish species larvae such as gadoid larvae (Last, 1978), red snapper, *Lutjanus campechanus* (Rabalais *et al*., 1980), walleye Pollock, *Theragra chalcogramma* (Dagg *et al*., 1984), turbot (Witt *et al*., 1984), mahimahi, *Coryphaena hippurus* (Kraul, 1991), the cod, *Gadus morhua* (van der Meeren & Naess, 1993), gilthead seabream, *Sparus aurata* (Fernandez *et al*., 1994), halibut, *Hippoglossus hippoglossus* (Harboe *et al*., 1994), mangrove snapper, *Lutjanus argentimaculatus* (Doi *et al*., 1994), grouper, *Epinephelus coioides* (Kohno *et al*., 1997; Su *et al*., 1997), dhufish, *Glaucosoma hebraicum* and pink snapper, *Pagrus auratus* (Payne *et al*., 2001). Due to both high nutritional value and high abundance in natural waters, the use of copepods as live feed for larval fish in hatcheries should be encouraged and developed.

It has been reported that generally, copepods feed on microalgal diets and bacteria (Treece, 2000). Copepods will response differently to microalgal diets which particularly

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influence copepods ability to produce eggs and viability of copepod's egg hatching rate (Ianora, 2005). In addition, there are numerous reports regarding the advantages and disadvantages of feeding copepods with particular microalgal species.

To increase fish seed production to support aquaculture in Indonesia, breakthroughs are required in the field and one of them is by introducing an intensive finfish hatchery program at the Research Institute for Mariculture Gondol, Bali by Danish Institute for Fisheries Research (DIFRES). The intensive hatchery has been equipped with seawater recycling system to meet with environmental safety and friendly standards. One of the introduced components was a harpacticoid copepod species, *Tisbe holothuriae* to produce nauplii for early feeding of produced finfish larvae (Steenfeldt *et al*., 2002).

The purpose of this experiment was to observe daily densities of harpacticoid copepod nauplii, *Tisbe holothuriae* and to find out their fatty acid compositions after fed with four different microalgal species i.e.: *Isochrysis tahiti Nannochloropsis oculata, Rhodomonas* sp., dan *Tetraselmis chuii* in laboratorial scale.

MATERIALS AND METHODS

Production of Microalgae

All four microalgal species of *I. tahiti*; *N. oculata, Rhodomonas* sp., dan *T. chuii* were produced in 80-L hanging carbuoys exposed to 40-W fluorescent lamps with average air tempaerature of about 24°C in an air conditioned controlled room. Medium used and operational culture procedure followed Steenfeldt *et al*. (2002).

Production of Copepod Nauplii

The experimental unit was a round conical bottom of 0.5 m^3 black fiberglass tank provided with arranged 60 m^2 polycarbonate substrate for adult harpacticoid copepod *T. holothuriae* to attach. Production method for copepod nauplii in the laboratory was carried out according to Steenfeldt *et al*. (2002).

Trials

Copepod nauplii were harvested from the original culture tanks through stratified filtration, pooled and counted according to volumetric sampling method. A number of adult copepods were stocked into prepared tanks together with the nauplii. The remaining adults were stocked back into their original culture tanks. The pooled nauplii and adult copepods were then fed with four microalgal diets at a density range of 1.0–12.0 x 10^5 cells/mL in one tank for 24 h before starting the trial to minimize bias due to their preference to a particular microalgal diet. Before stocking into twelve experimental units, those nauplii and adult copepods were counted and divided evenly.

The microalgal treatment diets were:

(A) I. tahiti;

(B) N. oculata;

(C) Rhodomonas sp., and

(D) *T. chuii*

Each treatment was in triplicates. Copepod nauplii were counted every morning during the trial while microalgae were added into each experimental unit in interval of 2–4 days. Water volume in each experimental tank was lowered through filtration before adding the microalgae. All nauplii and adult copepods retained in the filter were stocked back into their represented experimental tanks. Microalgae and copepod nauplii enumeration were conducted according to Boyd & Tucker (1992). Water quality observation was conducted twice a week i.e. dissolved oxygen and total ammonium, while water temperature and salinity were checked daily. The trial was carried out for 35 days.

All twelve experimental tanks were rinsed and dried well at the end of the trial. Copepod nauplii were separated with the adults by stratified filtration. The latter were cultured back for future use while the harvested nauplii samples were prepared according to modified procedures of Kates (1986) and Christie (1987) for further lipid and fatty acid analyses. Lipid extraction and fatty acid analysis were carried out at the Center for Food and Nutrition Laboratory of Gajah Mada University, Yogyakarta. Lipid extraction was conducted according to Soxhlet method while fatty acid analysis was carried out using a GC 14 B Shimadzu with 50-m capillary column CBP-10. Column temperature was set at 180°C–240°C with heating rate of 2.5°C/minute. Both injector and detector temperatures were 250°C with Helium gas as carrier at pressure of 180 kPa.

Detector used was FID (Flame Ionization Detector) integrated with Shimadzu C-RGA. Standard solutions used were Supelco® (U.S.A) Cat. # 47801 and Cat. # 178241A (for EPA: eicosapetaenoic acid and DHA: docosahexaenoic acid).

Observed values in this trial were expressed in average ± standard error. Data were analyzed in *t-test* of ANOVA with SAS® Version 6.12 for Windows® (SAS Institute, Inc., Cary, North Carolina, 1996). Least significant difference (LSD) was applied if significant differences among treatments were found (Sokal & Rohlf, 1981). Differences were assumed significant at a probability level, P = 0.05. Computer softwares used in data analysis and reporting were Microsoft® Excel XP and Microsoft Word XP.

RESULTS AND DISCUSSION

Average daily densities of harpacticoid copepod nauplii, *T. holothuriae* and microalgal in all treatments were depicted in Figure 1 and 2 while lipid and fatty acid compositions of copepod nauplii were shown in Table 1. Initial densities of stocked microalgae of *I. tahiti* (3.0–10.0 x 105 cells/mL) and *N. oculata* (10.0–40.0 x 105 cells/mL) were higher than that of the

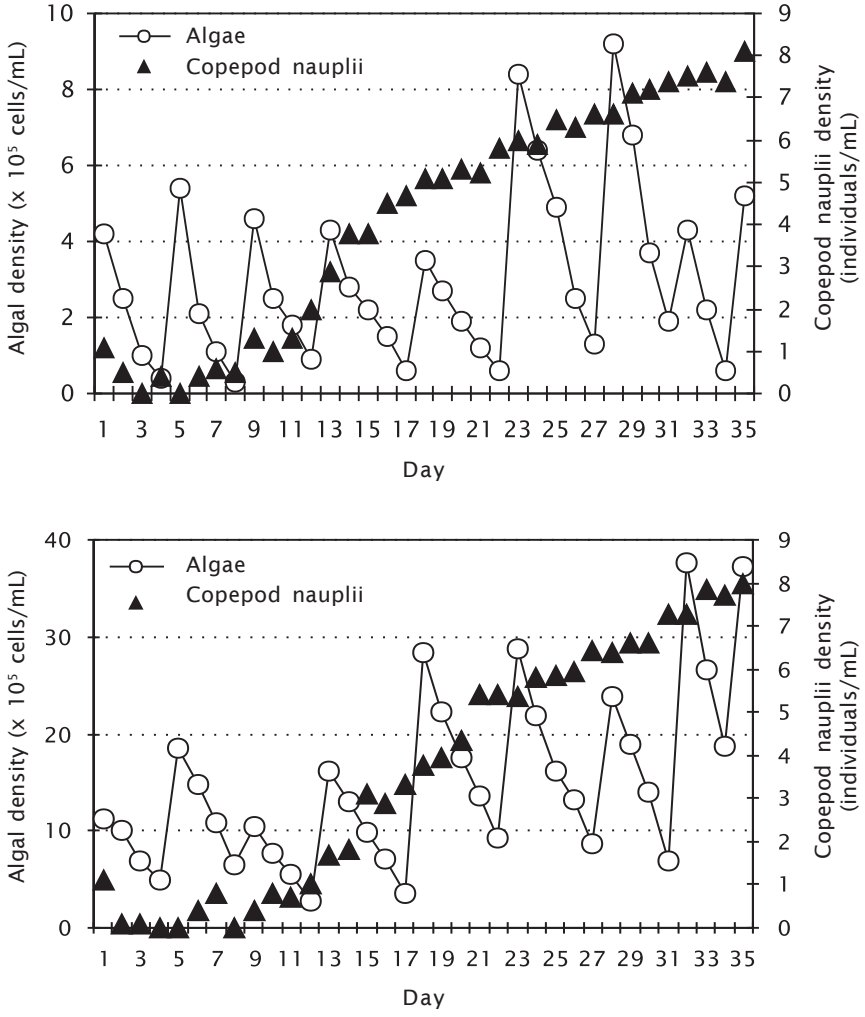

Figure 1. Daily average densities of harpacticoid copepod nauplii *T. holothuriae* fed with *I. tahiti* (top) and *N. oculata* (bottom)

other stocked microalgae species (1.0–3.0 x 105 cells/mL). Initial nauplii and adult copepod densities in each experimental unit were estimated at 1.1 ± 0.1 and 0.6 ± 0.0 individuals/mL, respectively while at the end of the trial, copepod nauplii densities were between 8 and 11 ind./mL. Copepod nauplii density elevated after 8–13 days of the trial. There were no significant differences of copepod nauplii densities among treatments at the end of the trial. However, there was significant differences in term of lipid content in copepod nauplii in this trial (P = 0.04). Lipid content of copepod nauplii fed with *T. chuii* (6.7%) was significantly higher (P = 0.03) compared to that of fed with *N. oculata*. Eicosapentaenoic acid (EPA) content was significantly higher (P = 0.02) in copepod nauplii fed with *N. oculata* (60.5 mg/100 g) but contained the lowest docosahexaenoic acid (DHA, 5.7 mg/100 g) resulted in the lowest DHA/EPA ratio of 0.1 (P = 0.02). DHA/EPA ratio is considered important because both fatty acids are essential fatty acids for marine finfish larvae (Sargent *et al.*, 1997). As reported by Kanazawa (1990) *in* Waspada *et al.* (1991) that DHA/EPA ratio in tiger grouper larvae (*Epinephelus fuscoguttatus*) was 5.8. In addition, requirement of essential

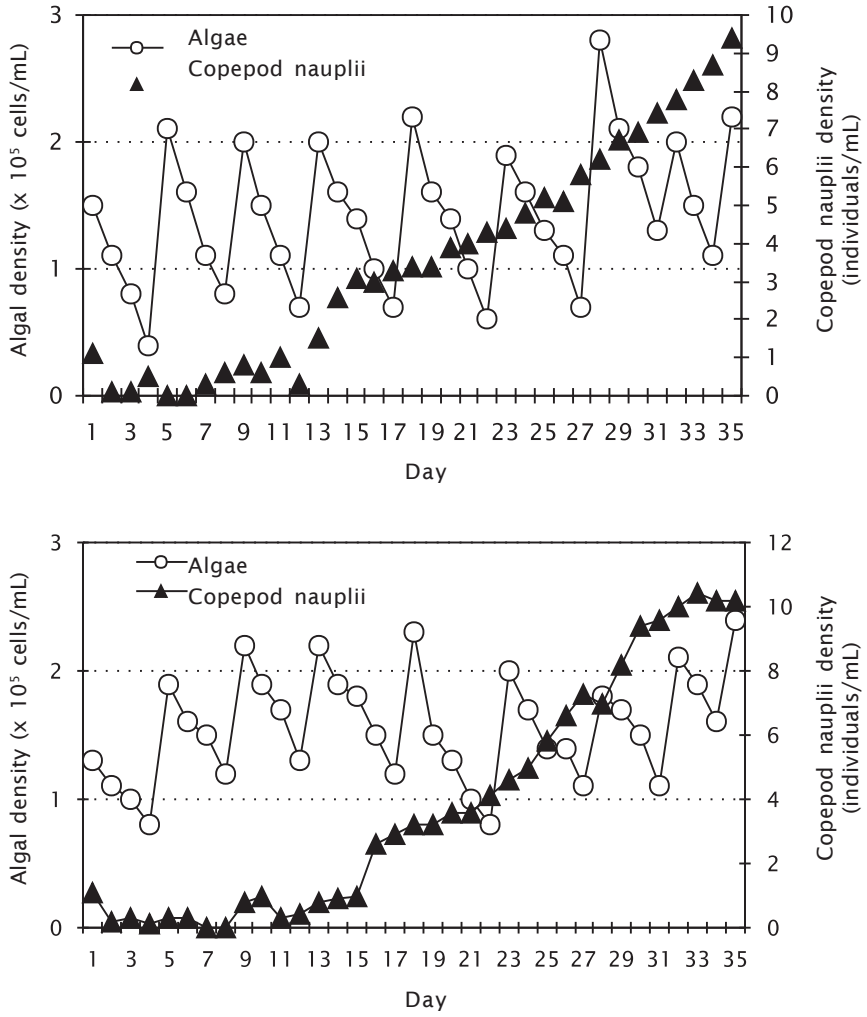

Figure 2. Daily average densities of harpacticoid copepod nauplii *T. holothuriae* fed with *Rhodomonas* sp. (top) and *T. chuii* (bottom)

Table 1. Fatty acid (mg/100 g dry weight) and lipid contents (% dry weight) of harpacticoid copepod nauplii *T. holothuriae* fed with four microalgal species

Fatty acids	Copepod nauplii fed with-			
	I. tahiti	*N. oculata*	*Rhodomonas* sp.	*T. chuii*
8:00		6.1		
12:00	7.3	9.2		9.2
14:00	12.2	18	23	23
14:1n-9c	7.2	7.7	14.6	10.7
16:00	177.2	285.9	142.7	338.5
16:1n-9c	94	143.5	107.4	143
17:00	33.5	50.2	90.2	28.2
18:00	41.8	22	24.6	39.4
18:01	281.7	239.3	325	706.3
18:2n-6c	231.7	459.5	367.5	439.9
18:3n-3	44.7	17.2	60.6	321.7
20:00	12.6	6.9		51
20:01	21.3	36.4	28.1	57.5
20:5n-3 (EPA)	18.9^{a}	60.5^{b}	47.0^{b}	42.5^{b}
22:6n-3 (DHA)	33.3^{a}	5.7^{b}	65.8^{c}	55.6^{ac}
DHA/EPA	1.8^{a}	0.1^{b}	1.4^{a}	1.3^{a}
Lipid (%)	4.6^{ab}	3.7^{a}	4.8^{ab}	6.7^{b}

1. Standard errors of average are not given for clarity of values
2. Values followed by the same superscript are not significantly different (P>0.05)

fatty acids corresponds to fatty acid contents in fish body. From the results, it can be seen that DHA/EPA ratios of copepod nauplii in all treatments were below the DHA/EPA ratio of tiger grouper larvae. The highest ration was found in copepod nauplii fed with *I. tahiti* (1.8).

Copepod nauplii fed with microalgae *I. tahiti* showed significantly lower lipid content (4.6%, P = 0.04) compared to the nauplii fed with microalgae *T. chuii* (6.7%). It has been widely known that microalgae *I. tahiti* contains essential fatty acids with high DHA/EPA ratio. Gimin (2004) reported lipid content of microalgae *I. tahiti* 23.7% and Anonimous (2006) reported 17% with DHA/EPA ratio of 4.1. However, this trial showed that high lipid content of microalgae did not affect lipid content of the copepod nauplii. As reported by Rhodes & Boyd (2005) that quality of copepod would not be affected by good microalgae as food. Fleeger (2005) reported that some harpacticoid copepods were able to synthesize longer-chained HUFA which will eventually increase the levels of n-3 fatty acids that are essential to fishes. It is also suggested that harpactioid copepods have enzymes (i.e. Δ-5, Δ-6 desaturase and elongase) that are necessary for the conversion of shorter chain n-3 poly-unsaturated fatty acids to the longer-chained essential fatty acids of DHA and EPA. On the other hand, Okauchi *et al*. (1990) reported that *N. oculata* had no DHA resulted in zero DHA/EPA ratio. This trial showed low content of DHA in copepod nauplii fed with *N. oculata*. Lipid content of copepod nauplii fed with microalgae *I. tahiti* was lower than that of fed with *T. chuii* but it had the highest DHA/EPA ratio compared to that of the other three treatments though did not differ significantly (P = 0.07) to that of fed with microalgae *Rhodomonas* sp. and *T. chuii*.

Reports on proximate analysis of copepod nauplii are scarcely available other than the general reports on proximate analysis of adult copepod from natural waters. Sumiarsa (2003) reported that lipid contents of cyclopoid copepod nauplii *Apocyclops panamensis* cultured in fertilized earthen ponds were between 5.7%–

7.8% with DHA/EPA ratios of 1.6–2.4 while the lipid contents of nauplii enriched with several commercial enriching agents were between 5.7–10.8 with DHA/EPA ratios of 1.0–3.3.

In natural temperate and tropical salt water systems, copepod abundance is generally low. Densities of 0.6 to 100 of calanoid, harpacticoid and cyclopoid copepod nauplii/L are commonly found in natural waters (Fukusho, 1991; Mishra & Panigrahy, 1996; Shansudin *et al*., 1997; Watanabe *et al*., 1998). In the present trial, final copepod nauplii densities were in between of that in natural waters between 8 and 10 ind./mL.

The feasibility of culturing copepods in laboratory environments has been investigated including intensive indoor systems (Stottrup *et al*., 1986; Vilela, 1992; Sun & Fleeger, 1995; Turk *et al*., 1998; Schipp *et al*., 1999). In laboratory trials where copepods have been produced in smaller volume systems, average densities of 185 calanoid copepod nauplii *Acartia* sp./L (Schipp *et al*., 1999) to 5,150 harpacticoid copepod nauplii *Tisbe holothuriae*/L (Stottrup *et al*., 1986) have been produced. Maximum density of 26,000 harpacticoid copepod nauplii *Tigriopus brevicornis*/L (Vilela, 1992) and 33,000 cyclopoid copepod nauplii *A. royi*/L (Cheng *et al*., 2001) have been reported in laboratory systems. However, intensive laboratory copepod production systems have been difficult to maintain over extended periods of time (Stottrup & Norsker, 1997).

Water quality parameters were in the ambient ranges for copepod culture i.e. temperatures were 22°C–24°C, dissolved oxygen were 3.5–4.1 mg/L, salinity was 34 ppt and total ammonia were 0.27–0.44 mg/L.

In general, all four microalgal species did not give any significant difference in final copepod nauplii density. However, lipid and fatty acid profiles of copepod nauplii fed with *N. oculata* showed inferior values compared to the other treatments.

CONCLUSION

With regard to the final quantity after seven weeks of trial, harpacticoid copepod *T. holothuriae* responded similarly to the four microalgal species, and there was no significant differences in the final nauplii density. Microalgal species could be fed based on their economical and conditional considerations. However, in term of lipid quality, harpacticoid copepod nauplii fed with *T. chuii* gave the highest lipid content although with inferior DHA/EPA ratio compared to the nauplii fed with *I. tahiti*. Feeding of high-lipid content microalgal species did not produce similar microalgal lipid levels in harpactiocid copepod nauplii in this trial. It is then suggested that further studies on microalgal species that can boost the lipid content in copepod nauplii have to be conducted.

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