Palm Oil-Based Enriched Diets for the Rotifer, *Brachionus plicatilis*, Improved the Growth of Asian Seabass (*Lates calcarifer*) Larvae

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Different types and inclusion levels of palm oil were incorporated in the enriched diets of L-type rotifer, *Brachionus plicatilis*, and fed to Asian seabass (*Lates calcarifer*) larvae. The dietary fish oil was replaced with either 50 or 75% of crude palm oil, CPO (CPO50, CPO75) and refined bleached deodorized palm olein, RPO (RPO50, RPO75). The enriched diet containing 100% fish oil (FO100) was used as the experimental control. Triplicate groups of the fish larvae of initial length 2.72 ± 0.14 mm were fed with enriched rotifer for 15 days. In general, palm oil-based enriched diets performed better than the control diet (FO100). Specifically, final mean body weight (31.3 ± 9.2 mg), final mean total length (11.5 ± 1.6 mm), SGR (29.0 ± 1.4%/day) and WG (7,769.4 ± 1,510.8%) of Asian seabass larvae fed RPO75 were significantly higher (*P* < 0.05) compared to those fed the other palm oil-based diet and FO100. The rotifer enriched with palm oil significantly affected the body proximate composition and fatty acid profiles of the fed larvae. The present study suggests that RPO and CPO can be considered as a good alternative dietary lipid for enrichment of rotifer to positively influence the nutritional requirements of the Asian seabass larvae and support their survival and growth.

**Keywords:** *Brachionus plicatilis*, palm oil, fish oil replacement, live feed, Asian seabass

**INTRODUCTION**

Asian seabass, *Lates calcarifer*, is a tropical marine fish that is widely farmed throughout Asia. Despite being an established aquaculture species, many obstacles remain in using an acceptable live feed in the first feeding of the larval rearing phase. Currently, rotifer (primarily the species, *Brachionus plicatilis*) is widely used as the first feed for Asian seabass larvae mainly because it is relatively easy to culture, highly digestible, rich in protein, and has been used as a vector for delivering compounds of diverse nutritional value to larval fish (Skallaia and Robin, 2004; Das et al., 2012; Dey et al., 2015). It is well-documented that live feed should be enriched prior to providing it to the larvae, with many studies focusing on the benefits of live feed enrichment using highly unsaturated fatty acids, HUFA (Rainuzzo et al., 1994; Sargent et al., 1999; Benitez-Santana et al., 2007, Tocher, 2010, Radhakrishnan et al., 2020) such as docosahexaenoic (DHA, 22:6n−3), eicosapentaenoic acid (EPA, 20:5n−3), and arachidonic acid (ARA, 20:4n−6) as the main energy sources and to ensure proper larval development (Rainuzzo et al., 1997; Sargent et al., 1999; Benitez-Santana et al., 2007, Tocher, 2010). Major dietary lipid source used in larviculture is fish-oil based emulsion through live feed enrichment (Rainuzzo et al., 1997). With the growth of aquaculture
industry the demand of fish oil for fish nutrition has increased (Alder et al., 2008; Nichols et al., 2010) resulting in shortages and threatening the sustainability of wild prey fish populations (Wassef et al., 2007). Fish oil substitution is, therefore, crucial to reducing the pressure on marine ecosystem while at the same time supporting the aquaculture industry (Tacon and Metian, 2008; Naylor et al., 2009; Alhazzaa, 2012). Developing aquafeeds by replacing fish oil and meal is an important area of research to pursue for reducing the pressure on forage fish. This will be among the major steps toward securing the sustainability of fed finfish aquaculture (Hua et al., 2019; Turchini et al., 2019).

Recent years have witnessed a heightened research interest in replacing fish oil with alternative lipid sources, mainly of plant origin. Palm oil is one of the vegetable oils that has received increasing attention in aquaculture feed research. It is abundantly available in several Asian countries. Apart from supplying dietary fat, palm oil is also a rich source of vitamin E, carotenoids, tocotrienols and natural antioxidants, and has a much cheaper price than the fish oil (Nasaretnam and Muhammad, 1993; Ng et al., 2007; Han et al., 2012; Singh et al., 2012). However, research on the use of palm oil as an alternative dietary fat source mostly involved juvenile and larger fish (Miller et al., 2008; Turchini et al., 2009; Torstensen and Tocher, 2010; Shapawi et al., 2011). In our earlier study based on juvenile stage of the Asian seabass (~5 g), the specimens fed diet based on RPO gained significantly higher (P < 0.05) weight than the other fish groups that were offered diets based on fish oil, soybean oil and canola oil at the end of feeding trials (Shapawi et al., 2011). Therefore, research is needed to investigate the potential of the abundantly available palm oil in the larval nutrition of Asian seabass to curtail the mortality and boost health and stamina of the most vulnerable phase of life of the fish. There is a glaring paucity of data on the application of edible plant-based oil enriched rotifer (Poh-Leong et al., 2012; Dhaneesh and Kumar, 2016; Campoverde and Eztevez, 2017), whereas the use of Artemia has received a great deal of attention in nutrition of fish larvae (Villalta et al., 2007; Arulvasu and Munuswamy, 2009; Hafezieh et al., 2010). The present study reports the results of experimental trials carried out for testing the effectiveness of the two different types of palm oils (CPO and RPO) as the lipid source in the enriched diets for the rotifer on Asian seabass larvae. To the best of our knowledge this is the first report on the replacement of fish oil with palm oil in the enriched diets for rotifers in the Asian seabass larvae. Other attempts to use palm oil as a substitute of fish oil have focused on juvenile and grow-out stages. For example, Wan et al. (2013) demonstrated that complete dietary fish oil replacement with either palm oil or lipid from poultry waste in barramundi (Lates calcarifer) juveniles (~3.6 g initial body weight) did not affect growth or hepato-somatic index. In our previous study on humpback grouper (Cromileptes altivelis) juveniles of about 10 g initial body weight, CPO and RPO were shown to have successfully replaced fish oil at 50% replacement level in a fish meal-based formulated diets without any detrimental effects on growth and survival (Shapawi et al., 2008). Similarly, growth performance of hybrid grouper (Epinephelus fuscoguttatus × E. lanceolatus) juveniles (~11 g initial body weight) fed CPO and RPO at 50% replacement level was slightly better than in those fed fish oil-based diet (Yong et al., 2019). Bell et al. (2002) have demonstrated that feeding diets containing palm oil (25 to 100% of added oil) to Atlantic Salmon (Salmo salar) juveniles (~55 g) had no significant effect on growth rate or feed conversion ratio, compared to fish fed 100% marine fish oil.

MATERIALS AND METHODS

Enriched Diet Preparation
A 2 × 3 factorial design using two types of palm oil (CPO and RPO) and three different levels (0, 50, and 75%) was applied to substitute fish oil in the enrichment diets for the rotifer. Fish oil-based enriched diet (FO100) was used as a control treatment. The oil emulsion was prepared by mixing 0.5 g of soy-lecithin and 10 g of egg albumin (Watanabe et al., 1989). Other ingredients included 2.1 g vitamin premix and 1.0 g mineral premix. The emulsion weighing 100 g was used for respective concentrations (50%, 75% and 100%) of either palm oil or fish oil in addition to 35.4% filtered seawater as shown in Table 1. The oil emulsion was homogenized using IKA homogenizer (T-25 basic Ultra Turrax) at 14,000 rpm/minute.

Rotifer Culture and Enrichment Protocol
The L-type rotifer was obtained from Fish Hatchery of Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah (UMS). The rotifers were cultured in 150 L conical fiber reinforced plastic (FRP) tank with the seawater volume of 100 L at the density of 600 rotifers/ml. The salinity and temperature of water were maintained at 30 ppt and 28.59 ± 0.16°C, respectively. Individual rotifer tanks were well-aerated (800 ml/second). Rotifers were enriched using fortified diets in a concentration of 0.5 g/L for 24 h. After this treatment, the enriched rotifers were harvested by using a 60 μm—nylon mesh screen, washed in de-chlorinated seawater and then used for larval feeding at the density of 20 individual/ml. A sample was stored for subsequent chemical analysis.

Proximate and Fatty Acid Analysis
The enriched diets, rotifer and larval whole-body proximate composition were analyzed by methods suggested by the Association of Official Analytical Chemists (AOAC, 1997). The enrichment diets and enriched rotifer were subjected to lipid extraction by using chloroform: methanol (1:1, v/v) following Bligh and Dyer (1959) method. The lipid extract was then fractionated by a short column filled with silica gel 60 F254 (Merck, Darmstadt, Germany) with a mesh size of 0.063–0.2 mm in a hexane: ethyl acetate solvent system (9:1, v/v). Methylation of the extract was carried out inside a reaction vessel for 2 h using sodium methylate. The extract was purified using the silica gel column system before the fatty acid methyl esters were analyzed in a gas chromatograph (Shimadzu GC-2010, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector and an auto injector. The esters were separated using a capillary column (60 m × 0.25 mm ID; BPX70 column, SGE Australia Pty. Ltd., Ringwood, Vic., Australia) and chromatograph peaks were identified by comparing retention...
### TABLE 1 | Ingredients and proximate composition of different enrichment diets (g/100 g).

| Ingredients | FO100 | CPO50 | CPO75 | RPO50 | RPO75 |
|-------------|-------|-------|-------|-------|-------|
| Fish oil    | 50.0  | 25.0  | 15.0  | 25.0  | 15.0  |
| RBDPO       | /     | /     | /     | 25.0  | 35.0  |
| CPO         | /     | 25.0  | 35.0  | /     | /     |
| Vitamin premix | 2.1  | 2.1   | 2.1   | 2.1   | 2.1   |
| Mineral premix | 1.0  | 1.0   | 1.0   | 1.0   | 1.0   |
| Soy-lecithin | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   |
| Egg albumin | 10.0  | 10.0  | 10.0  | 10.0  | 10.0  |
| Filtered sea water | 36.4 | 36.4  | 36.4  | 36.4  | 36.4  |
| Total       | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

**Component**

| Proximate composition (%DM) | FO100 | CPO50 | CPO75 | RPO50 | RPO75 |
|-----------------------------|-------|-------|-------|-------|-------|
| Moisture                    | 39.6 ± 0.1 | 40.7 ± 0.4 | 41.4 ± 0.2 | 41.4 ± 0.2 | 39.0 ± 0.1 |
| Lipid                       | 50.3 ± 2.8 | 51.5 ± 1.3 | 50.7 ± 1.0 | 51.3 ± 0.9 | 51.5 ± 2.2 |
| Protein                     | 1.6 ± 0.2  | 1.9 ± 0.3  | 1.7 ± 0.1  | 1.5 ± 0.4  | 1.8 ± 0.1  |
| Ash                         | 4.2 ± 0.4  | 4.5 ± 0.2  | 4.6 ± 0.5  | 4.1 ± 0.2  | 4.2 ± 0.3  |

**a,b** Sawit Kinabalu Sdn. Bhd. (Lumadan Palm Oil Mill, Beaufort, Sabah).

**c** Vitamin premix (Dexchem Industries Sdn. Bhd.), contains (kg-1 dry weight): ascorbic acid 45 g; inositol 5 g; choline chloride 75 g; niacin 4.5 g; riboflavin 1 g; pyridoxine HCl 1 g; thiamine HCl 0.92 g; dicalciumpantothenate 3 g; retinyl acetate 0.6 g; menadione 0.02 g; folic acid 0.09 g; vitamin B12 0.001 g; cellulose.

**d** Mineral premix (Dexchem Industries Sdn. Bhd.), contains (kg-1 dry weight): calcium phosphate monobasic 270.98 g; calcium lactate 327 g; ferrous sulphate 25 g; magnesium sulphate 132 g; potassium chloride 0.15 g; copper sulphate 0.785 g; manganese oxide 0.8 g; cobalt carbonate 1 g; zinc oxide 3 g; sodium selenite 0.011 g; calcium carbonate 129.27 g.

**e** Xi’an YuenSun Biological Technology Co., Ltd. (China Manufacturer).

**f** STF Agriculture Sdn. Bhd.

### TABLE 2 | Growth performance of Asian seabass larvae fed different types of enriched rotifers.

| Enriched diet (Palm oil type/level) | Final BW (mg) | Final TL (mm) | SGR (% mg day$^{-1}$) | WG (%) |
|------------------------------------|---------------|---------------|------------------------|--------|
| FO100                              | 9.4 ± 8.8$^{c}$ | 7.3 ± 0.8$^{d}$ | 21.0 ± 0.5$^{c}$ | 2,233.3 ± 180.9$^{c}$ |
| CPO50                              | 23.0 ± 10.0$^{c}$ | 10.3 ± 1.7$^{c}$ | 26.9 ± 1.2$^{ab}$ | 5,656.3 ± 1,033.8$^{c}$ |
| CPO75                              | 17.3 ± 8.9$^{c}$ | 9.5 ± 1.5$^{c}$ | 24.8 ± 1.8$^{ab}$ | 4,110.4 ± 1,073.1$^{b}$ |
| RPO50                              | 11.1 ± 3.8$^{c}$ | 8.7 ± 1.3$^{c}$ | 21.7 ± 2.0$^{c}$ | 2,587.5 ± 795.6$^{c}$ |
| RPO75                              | 31.3 ± 9.2$^{c}$ | 11.5 ± 1.6$^{a}$ | 29.0 ± 1.4$^{a}$ | 7,769.4 ± 1,510.8$^{a}$ |

**One-way ANOVA**

| Parameter | Palm oil type | Palm oil level | Oil type $\times$ Oil level |
|-----------|--------------|----------------|-----------------------------|
| Final BW (mg) | 0.663 | 0.000 | 0.000 |
| Final TL (mm) | 0.624 | 0.000 | 0.000 |
| SGR (% mg day$^{-1}$) | 0.621 | 0.000 | 0.000 |
| WG (%) | 0.662 | 0.000 | 0.000 |

**Initial body weight = 0.4 ± 0.1 mg; Initial total length = 2.7 ± 0.1 mm.**

**Mean values with same superscripts in the same column are not significantly different between the treatments (Duncan’s multiple range test, P > 0.05).**

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**Experimental Design and Feeding Trial**

Asian seabass larvae were obtained from the Fish Hatchery of BMRI (UMS). A total of 54,000 newly hatched larvae with an initial weight and length of 0.44 ± 0.11 mg and 2.72 ± 0.14 mm, respectively were observed for general health condition and held in the tanks for experimental trials. Triplicate groups of larvae were randomly distributed into 18 conical FRP tanks (150 liters) with initial stocking density of 30 individual per liter. The experimental tanks were randomly arranged in triplicate and

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*times with known standards (Supelco™ 37 Component FAME mix, Supelco Inc., Bellefonte, USA).*

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in closed system, and supplied with continuous mild aeration (250 ml/second). Starting from 36 h after hatching (hAH), the respective batches of enriched rotifer were fed to the fish larvae at the density of 20 individual/ml. *Nannochloropsis oculata* was also provided in all the tanks at concentration of 0.5 × 10^6 cell/ml. There was no cleaning for up to 5 days to reduce the disturbance to the larvae. After 5 days of rearing, water was routinely changed (5%) to avoid accumulation of metabolic wastes. The density of enriched rotifer was monitored under a profile projector (Mitutoyo, PJ-A3000, Japan) twice daily at 0830 am and 1400 pm. The physiochemical parameters of water temperature, dissolved oxygen and pH throughout the experiment were 29.31 ± 0.04, 8.12 ± 0.05 and 7.9 ± 0.05, respectively. The experimental duration was 15 days.

### Performance Evaluation

Triplicate pooled sample of initial stock and 5 days after hatching (dAH)-larvae (n = 100) and 10 dAH and 15 dAH-larvae (n = 50) were measured using an analytical balance. At every 5 days interval up to 15 dAH, 10 larvae (n = 10) from each treatment were sampled, anesthetised with Trasmore (alpha-methylquinoline) (Nika Trading, Puchong, Malaysia) and measured under a profile projector (Mitutoyo, PJ-A3000, Japan) for morphometric observations on total length (TL), standard length (SL), body height (BH), muscle height (MH), head length (HL), head depth (HD), gut height (GH), and eye diameter (ED). The larvae were examined under a light microscope (Nikon, Eclipse E600, Japan) for rotifer ingestion and gut observation. The condition factor and ratio of total length to body weight (CF) (Chatain, 1994) were determined at the end of the experiment.

Other parameters were calculated using the following formulas:

- Specific growth rate (SGR, %/d) = [(In final weight, mg ln initial weight, mg)days] × 100
- Survival (%) = [(Initial fish number, final fish number) Initial fish number] × 100
- Condition factor (CF) = [fish weight(total length)] × 100
- Total length/ body weight (TL/W) ratio = [(total length, mm)(body weight, mg)]
- Weight gain, WG (%) = [(final body weight, g initial body weight, g] × 100.

### Statistical Analysis

IBM Statistical Package of Social Sciences Version 23.0 was used for all the statistical analyses. All the data in percentage were arcsine transformed before the analysis. The quantitative data was subjected to one-way analysis of variance (ANOVA) to determine the mean differences among the treatments at 0.05 significance level. The homogeneity of variance was tested using Levene’s test and multiple comparisons among treatments were presented using a Duncan’s multiple range test. Effects of oil types and levels, and their interactions were determined by two-way ANOVA.

### RESULTS

#### Growth Performance

Data on larval growth performance is presented in Table 2. Final mean body weight (31.3 ± 9.2 mg), final mean total length (11.5 ± 1.6 mm), SGR (29.0 ± 1.4%/day) and WG (7,769.4 ± 1,510.8%) of Asian seabass larvae fed RPO75 were significantly higher (P < 0.05) compared to those fed the other palm oil-based diet and FO100. The palm oil inclusion level significantly affected the survival rate and variation (%) of the larvae in the 15 day experimental period. The different batches of enriched rotifer did not show any significant effect on 5 and 10 dAH. However, at the end of the experiment (15 dAH), the survival was significantly affected by palm oil level. CPO50 yielded higher survival (77.4 ± 9.4%) compared to FO100 (54.5 ± 2.0%) and RPO-based treatments (61.2 ± 8.7–67.8 ± 12.3%).

### Morphometric Variables and Body Condition

The morphometric variables and body indices of Asian seabass at the end of the experiment are presented in Table 4. The morphometric value of each parameter was affected by palm oil level. RPO75 shows significantly higher (P < 0.05) SL, MH, ED (10.0 ± 1.0, 2.6 ± 0.5 and 1.0 ± 0.1, respectively). The lowest (P < 0.05) value of HL, HD and ED (2.5 ± 0.2, 1.9 ± 0.2 and 0.7 ± 0.1, respectively) was observed in fish fed FO100 compared to the other treatments. SL, BH, MH, GH (6.4 ± 0.8, 2.1 ± 0.2, 1.6 ± 0.1, 0.4 ± 0.1, respectively) of FO100 treatment were significantly lower than the other ones, except RPO50. Meanwhile, the condition factor was not significantly affected by palm oil type and level. However, T:W was significantly influenced by oil type only. TL:W was significantly higher (P < 0.05) in FO100 and RPO50 compared to the other treatments.

### Proximate Composition of Enriched Rotifer and Larval Fish

Table 5 shows the proximate composition of enriched rotifer. The proximate composition of enriched rotifer was not significantly affected by palm oil type and level except for protein.
TABLE 4 | Morphometric variables and body indices of Asian seabass larvae fed different types of enriched rotifers.

| Enriched diet | Parameters       | SL ± SD | BH ± SD | MH ± SD | HL ± SD | HD ± SD | GH ± SD | ED ± SD | K ± SD | TL:W ± SD |
|---------------|-----------------|---------|---------|---------|---------|---------|---------|---------|--------|-----------|
| One-way ANOVA |                 |         |         |         |         |         |         |         |        |           |
| FO100         |                 | 6.4 ± 0.8 c | 2.1 ± 0.2 d | 1.6 ± 0.1 c | 2.5 ± 0.2 d | 1.9 ± 0.2 c | 0.4 ± 0.1 c | 0.7 ± 0.1 c | 3.4 ± 0.4 | 0.8 ± 0.3 c bc |
| CPO50         |                 | 8.9 ± 1.3 b | 3.2 ± 0.4 ab | 2.1 ± 0.5 b | 3.5 ± 0.6 ab | 2.5 ± 0.3 b | 0.6 ± 0.2 b | 0.9 ± 0.1 b | 3.2 ± 0.4 | 0.5 ± 0.1 a |
| CPO75         |                 | 8.1 ± 1.3 b | 3.0 ± 0.4 b | 2.0 ± 0.3 bc | 3.2 ± 0.5 bc | 2.3 ± 0.3 bc | 0.7 ± 0.1 ab | 0.8 ± 0.1 ab | 3.0 ± 0.4 | 0.6 ± 0.2 ab |
| RPO50         |                 | 7.8 ± 1.0 c | 2.6 ± 0.5 a | 2.0 ± 0.4 ab | 3.0 ± 0.4 c | 2.2 ± 0.3 c | 0.5 ± 0.1 c | 0.8 ± 0.1 b | 2.9 ± 0.2 | 0.9 ± 0.2 c |
| RPO75         |                 | 10.0 ± 1.0 b | 3.5 ± 0.5 a | 2.6 ± 0.5 a | 3.9 ± 0.5 a | 2.8 ± 0.2 a | 0.8 ± 0.2 a | 1.0 ± 0.1 a | 3.1 ± 0.3 | 0.4 ± 0.1 a |
| Two-way ANOVA (p-value) | |         |         |         |         |         |         |         |        |           |
| Palm oil type |                 | 0.949 ± 0.658 | 0.125 ± 0.072 | 0.427 ± 0.511 | 0.037 ± 0.597 | 0.015 ± 0.138 |
| Palm oil level|                 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.048 ± 0.138 |
| Oil type × oil level | | 0.000 ± 0.001 | 0.007 ± 0.001 | 0.001 ± 0.001 | 0.013 ± 0.006 | 0.534 ± 0.142 |

Means with same superscripts in the same column are not significant differences between treatment (Duncan’s multiple range test, P > 0.05).

content that was markedly influenced by palm oil type (P = 0.005). RPO50-enriched rotifer contained significantly (P < 0.05) higher protein (55.2 ± 0.3%) compared to the other treatments. Meanwhile, the whole-body proximate composition of Asian seabass was not significantly affected by the enriched diet. Fish fed RPO-enriched diet resulted in slightly higher protein and lipid contents compared to the other treatments as shown in Table 6.

**Fatty Acid Composition of Enriched diets, Rotifer, and Larval Fish**

In general, the replacement of fish oil with palm oil (CPO and RPO) increased the C:16 (palmitic acid), C18:1n9 (oleic acid) and reduced total PUFA and n-3HUFA, especially DHA content of the enriched diets. The total saturates, monoenes and total PUFA were affected by palm oil level and n-3 and n-3/n-6 were affected by the palm oil type and level (Table 7). The total saturated fatty acids in palm oil-based enriched rotifer were significantly (P < 0.05) higher than in the control diet, ranging from 39.0 ± 0.1% to 40.9 ± 0.1% (Table 8). The replacement of fish oil with dietary palm oil had significantly affected the level of saturates and monoenes of the enriched rotifers. The total PUFA, n-3 PUFA, n-3/n-6, EPA and DHA levels were significantly higher in FO100 than in other groups. The total fatty acids of enriched rotifer were significantly affected by palm oil type and level. Table 9 shows the whole body fatty acid composition of Asian seabass at the end of experiment. Total fatty acids of Asian seabass body composition yielded the same pattern as in the enriched diet and rotifer. The palm oil-based diets contained significantly (P < 0.05) higher level of C:16 and C18:1n9 compared to FO100. Overall, the total fatty acids of palm oil enriched diets, rotifers and Asian seabass larvae were significantly affected by the palm oil type and level except total PUFA and n-3 which showed no significant effect by the palm oil type.
TABLE 5 | Proximate composition (% dry weight basis) of enriched rotifer.

| Enriched diets (Palm oil type/level) | Parameters | Moisture | Protein | Lipid | Ash |
|-------------------------------------|------------|----------|---------|-------|-----|
| FO100                               |            | 89.3 ± 0.4 | 51.3 ± 1.6<sup>ab</sup> | 24.4 ± 3.7 | 6.4 ± 0.6 |
| CPO50                               |            | 89.9 ± 0.3 | 49.8 ± 5.5<sup>ab</sup> | 22.6 ± 4.0 | 6.3 ± 1.0 |
| CPO75                               |            | 89.8 ± 0.1 | 45.9 ± 0.3<sup>b</sup> | 20.4 ± 1.9 | 5.8 ± 0.4 |
| RPO50                               |            | 89.7 ± 0.3 | 55.2 ± 0.3<sup>a</sup> | 21.7 ± 2.6 | 6.2 ± 0.7 |
| RPO75                               |            | 89.9 ± 0.1 | 53.6 ± 2.7<sup>a</sup> | 23.3 ± 3.9 | 6.3 ± 0.7 |

Two-way ANOVA

Palm oil type 0.879 0.005 0.686 0.669
Palm oil level 0.878 0.252 0.358 0.680
Palm oil type x oil level 0.764 0.072 0.624 0.751

Means with same superscripts in the same column are not significant differences between treatment (Duncan's multiple range test, P > 0.05).

DISCUSSION

Lipid is the main energy source for marine fish, especially in the early stage of its life. Short-term enrichment of rotifer using fish oil emulsion results in lipid-encapsulated rotifers rich in EPA and DHA. Generally, this is a nutritionally stable composition and makes the diet palatable which is important especially for fish larvae (Dhert et al., 2001). In the present study, Asian seabass larvae achieved faster growth by ingesting the palm-oil enriched rotifer, indicating the ability of the larvae to utilize the available lipid for energy consumption and growth. Fish fed palm-oil-based enriched rotifer showed highest growth performance compared to control diet. It is well-documented that palm oil is characterized by high level of β-carotene and is a rich source of antioxidants. These components are known to be excellent natural free radical acceptors and may exert beneficial effects on growth when fish are fed high levels of palm oil (Lim et al., 2001; Ng et al., 2003). A study by Jalali et al. (2008) emphasized that supplementation of antioxidants is necessary when high levels of n-3 PUFA are incorporated in larval diets to avoid adverse effects and to improve larval performance on beluga (Huso huso) larvae. Investigations conducted by Tocher et al. (2003) have shown that supplementation of α-tocopherol, which is present in palm oil, improved the growth and reduced the lipid peroxidation in sea bream and turbot (Psetta maxima) tissues. Betancor et al. (2013) observed that the elevation of dietary vitamin E levels in the palm oil markedly reduced the incidence of these symptoms and increased the tissue content of several PUFA, and improved the growth in European sea bass. Espe et al. (2007) stated that feeding lower level of fish oil than plant oil improved n-3 HUFA retention efficiency. However, inclusion of high level of fish oil in the enriched diet resulted in decline in growth of Asian seabass larvae, mainly due to the poor retention efficiency of fatty acids (Bell et al., 2002; Espe et al., 2007). Lipid oxidation impairs the nutritional value of animal products due to high proportion of unsaturated fatty acids. This is consistent with the findings reported by Jalali et al. (2010) and Babalola et al. (2013). Studies on the use of palm oil in enriching the live feed, particularly rotifer, are limited and the information is scarce on marine fish larvae. Poh-Leong et al. (2012) used palm oil mill effluent (POME)-enriched bacteria as food for rotifer and fed it to marble goby, Oxyeleotris marmorata. The study reported that the POME significantly enhanced the rotifer production, and improved growth and survival. Concordant findings were also obtained on other plant oil-based enriched live foods for different species (Smith et al., 2004; Menoyo et al., 2007; Huang et al., 2008; Hafezieh et al., 2010; Tehrani et al., 2014; Kazemi et al., 2016).

The morphological features (phenotypic characters), CF and TL/W ratio that are normally used as indicators of nutritional status of fish larvae showed that the Asian seabass larvae were developing normally based on the consistent pattern in all the treatment groups. During the larval phase, survival appeared to be strongly influenced by the energy status of the larvae. Thus, the high levels of fatty acids in the live prey are essential to satisfy the elevated energy demand and to promote growth (Dhanesh and Kumar, 2016). Current study shows that the survival is affected by palm oil level and fatty acid composition particularly DHA and EPA. Rich amounts of these substances in the control treatment significantly reduced the survival. A study conducted by Planas and Cunha (1999) also suggested that higher levels of DHA or n-3 HUFA reduced the survival of marine fish larvae. Izquierdo et al. (1992) demonstrated that in the larvae of Japanese, P. olivaceus, DHA content in Artemia metanauplii did not affect the survival rate but influenced the size when the larvae were fed with artemia containing high level of DHA. Thus, the requirement of dietary DHA level of marine finfish larvae is species dependant as highlighted by Sargent et al. (1999).

The fatty acid composition of enriched rotifer and Asian seabass larvae reflected the fatty acid profile in the enriched diets. Earlier studies have demonstrated that fatty acid composition of enriched rotifer is a reflection of its enrichment and, therefore, it depends on the culture condition and presence of nutrients (Kotani et al., 2013; Hamre, 2016). Specifically, the fish oil replacement at 50% with CPO and 75% with RPO performed...
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Table 7: Fatty acid composition (% of total fatty acids) of enriched diets.

| Fatty acid | FO100 | CPO50 | CPO75 | RPO50 | RPO75 |
|------------|-------|-------|-------|-------|-------|
| C14:0      | 3.6±0.8 | 3.3±0.6 | 2.5±0.1 | 3.3±0.1 | 1.7±0.2 |
| C14:1      | 0.8±0.4 | 0.5±0.2 | 0.5±0.0 | 0.7±0.2 | 0.6±0.1 |
| C15:0      | 0.5±0.4 | 0.4±0.2 | 0.3±0.0 | 0.3±0.2 | 0.3±0.1 |
| C16:0      | 12.7±0.1 | 24.8±0.5 | 15.4±0.1 | 24.3±0.5 | 16.9±0.1 |
| C16:1      | 9.7±0.2 | 5.3±0.1 | 4.7±0.1 | 4.4±0.1 | 2.9±0.1 |
| C17:0      | 0.4±0.3 | 0.4±0.2 | 0.3±0.0 | 0.4±0.2 | 0.4±0.0 |
| C18:0      | 2.6±0.2 | 5.1±0.1 | 2.5±0.1 | 4.2±0.1 | 2.6±0.1 |
| C18:1n9    | 15.8±0.4 | 34.5±0.2 | 43.6±0.2 | 41.3±0.9 | 41.3±0.4 |
| C18:2n6    | 5.6±0.2 | 0.2±0.0 | 0.2±0.0 | 0.3±0.0 | 0.5±0.0 |
| C18:3n3    | 3.5±0.1 | 3.2±0.0 | 10.6±0.2 | 3.8±0.1 | 9.7±0.1 |
| C20:1      | 0.4±0.2 | 3.3±0.3 | 2.6±0.4 | 2.1±0.3 | 1.3±0.0 |
| C20:3n6    | 0.3±0.2 | 0.8±0.1 | nd | 0.9±0.1 | nd |
| C20:4n6    | 2.6±0.5 | 0.5±0.0 | 0.5±0.0 | 0.4±0.1 | 0.9±0.0 |
| C22:1n9    | 0.7±0.0 | 1.4±0.0 | 0.9±0.0 | 0.7±0.0 | 0.7±0.0 |
| C20:5n3    | 15.7±0.1 | 7.9±0.4 | 7.0±0.1 | 5.1±0.4 | 7.9±0.3 |
| C22:6n3    | 20.4±0.2 | 10.3±0.5 | 7.4±0.1 | 6.4±0.3 | 10.6±0.2 |

Enriched diets (Palm oil type/level)

| Parameters | Total saturates | Total monoenes | Total PUFA | n-3 | n-3/n-6 | DHA/EPA |
|------------|----------------|----------------|------------|-----|--------|---------|
| One-way ANOVA | FO100 | 19.9±0.2 | 30.8±0.6 | 49.3±0.4 | 37.1±0.3 | 5.6±0.1 | 1.3±0.0 |
|             | CPO50 | 34.2±0.5 | 43.0±0.4 | 18.5±0.9 | 18.4±0.4 | 4.4±0.3 | 1.3±0.0 |
|             | CPO75 | 21.4±0.3 | 52.2±0.3 | 26.4±0.5 | 15.0±0.4 | 1.3±0.0 | 1.1±0.0 |
|             | RPO50 | 33.0±0.7 | 12.4±1.0 | 17.6±0.5 | 12.0±0.4 | 2.3±0.1 | 1.3±0.1 |
|             | RPO75 | 22.4±0.1 | 47.2±0.3 | 30.4±0.3 | 19.3±0.4 | 1.8±0.1 | 1.3±0.0 |
| Two-way ANOVA (p-value) | Palm oil type | 0.057 | 0.112 | 0.125 | 0.012 | 0.000 | 0.007 |
|             | Palm oil level | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 |
|             | Oil type x oil level | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

RPO75 enriched rotifer showed a significant correlation between the significantly high growth performance and DHA:EPA ratio (1.4) compared to other palm oil-based treatments. Current study further showed that high dietary DHA level did not result in any significant growth benefit to the larvae. However, the nutritional requirements for DHA and EPA have been found to be both species-specific as well as specific to the developmental stage of the larvae (Copeman et al., 2002; Villalta et al., 2005).

The fatty acid composition of the diets was clearly reflected in the enriched rotifer and body fatty acid composition of Asian seabass larvae. However, n-3 HUFA levels in fish body fed RPO75 and CPO50 showed inconsistent profile where n-3 HUFA levels were higher (9.9 and 11.4%, respectively) which contrasted with the supplied enriched rotifer (6.5 and 8.1%, respectively). Findings of Tocher et al. (2003) and Mohd-Yusof...
TABLE 8 | Fatty composition (percentage of total fatty acids) of enriched rotifers.

| Fatty acid (One-way ANOVA) | Enriched diets |
|---------------------------|----------------|
|                           | FO100          | CPO50          | CPO75          | RPO50          | RPO75          |
| C14:0                     | 3.4 ± 0.1      | 2.8 ± 0.0      | 4.1 ± 0.1      | 3.1 ± 0.1      | 1.8 ± 0.0      |
| C15:0                     | 0.5 ± 0.0      | 0.2 ± 0.0      | 0.3 ± 0.0      | 0.2 ± 0.0      | 0.2 ± 0.0      |
| C16:0                     | 22.4 ± 0.3     | 32.0 ± 0.0     | 29.8 ± 0.0     | 32.1 ± 0.2     | 34.1 ± 0.6     |
| C16:1                     | 7.7 ± 0.8      | 2.9 ± 0.0      | 4.7 ± 0.1      | 3.6 ± 0.2      | 1.9 ± 0.6      |
| C17:0                     | 0.4 ± 0.0      | 0.2 ± 0.0      | 0.2 ± 0.0      | 0.2 ± 0.0      | 0.2 ± 0.0      |
| C18:0                     | 4.6 ± 0.1      | 4.7 ± 0.0      | 4.7 ± 0.0      | 4.8 ± 0.0      | 4.79 ± 0.0     |
| C18:1n9                   | 24.3 ± 0.2     | 42.1 ± 0.1     | 34.9 ± 0.3     | 39.9 ± 0.3     | 46.7 ± 0.1     |
| C18:2n6                   | 2.9 ± 0.1      | 5.5 ± 0.1      | 4.4 ± 0.1      | 4.3 ± 0.4      | 5.8 ± 0.1      |
| C20:0                     | 0.3 ± 0.0      | 0.3 ± 0.0      | 0.3 ± 0.0      | 0.3 ± 0.0      | 0.3 ± 0.0      |
| C20:4n6                   | 0.9 ± 0.0      | 0.3 ± 0.0      | 0.5 ± 0.0      | 0.3 ± 0.0      | 0.3 ± 0.0      |
| C22:1n9                   | 4.0 ± 0.3      | 1.2 ± 0.0      | 2.1 ± 0.1      | 1.4 ± 0.0      | 0.6 ± 0.0      |
| C20:5n3                   | 7.7 ± 0.1      | 4.9 ± 0.0      | 8.4 ± 0.1      | 6.0 ± 0.1      | 2.7 ± 0.0      |
| C22:6n3                   | 10.2 ± 0.3     | 3.1 ± 0.0      | 5.6 ± 0.1      | 3.9 ± 0.1      | 3.8 ± 0.1      |

2 Enriched diets (Palm oil type/level)

| Parameters | Total saturates | Total monoenes | Total PUFA | n-3 | n-3/n-6 | DHA/EPA |
|------------|----------------|----------------|------------|-----|---------|---------|
| (One-way ANOVA) |                |                |            |     |         |         |
| FO100      | 31.6 ± 0.2     | 36.0 ± 1.2     | 21.7 ± 0.5  | 17.9 ± 0.4 | 4.7 ± 0.0 | 1.3 ± 0.0 |
| CPO50      | 39.8 ± 0.1     | 46.2 ± 0.2     | 13.8 ± 0.2  | 8.1 ± 0.1  | 1.4 ± 0.0 | 0.6 ± 0.0 |
| CPO75      | 39.0 ± 0.1     | 41.7 ± 0.5     | 18.8 ± 0.3  | 14.0 ± 0.2 | 2.9 ± 0.0 | 0.7 ± 0.0 |
| RPO50      | 40.2 ± 0.3     | 44.9 ± 0.5     | 14.5 ± 0.6  | 9.9 ± 0.2  | 2.1 ± 0.2 | 0.7 ± 0.0 |
| RPO75      | 40.9 ± 0.1     | 49.2 ± 0.2     | 12.6 ± 0.2  | 6.5 ± 0.1  | 1.1 ± 1.4 | 1.4 ± 0.6 |
| Two-way ANOVA (p-value) |                |                |            |     |         |         |
| Palm oil type | 0.000          | 0.000          | 0.000      | 0.000 | 0.000   | 0.000   |
| Palm oil level | 0.000          | 0.000          | 0.000      | 0.000 | 0.000   | 0.000   |
| Oil type × oil level | 0.000         | 0.000          | 0.000      | 0.000 | 0.000   | 0.000   |

1 Minor fatty acids (<0.2% from total fatty acids) were not included in the table.

Means with same superscripts in the same 1 row and 2 column are not significant differences between treatment (Duncan’s multiple range test, P > 0.05).

et al. (2010) have revealed that Asian seabass was able to maintain levels of DHA above the dietary level and attributed it to the selective retention of these nutrients in the palm oil linked to the conversion of EPA to DHA. This study demonstrated that the nutritional value of rotifer was not influenced by the enrichment diets except the protein level. RPO-enriched rotifer showed higher protein level compared to the other treatments. The mean protein level in rotifiers varied in the range of 45.9–55.2%. Generally, protein level in rotifiers are relatively stable at ~34–52% as reported by Caric et al. (1993) and Hamre et al. (2013); Hamre (2016). Slight changes in lipid level in rotifiers have been observed to affect the dry matter protein content (Srivastava et al., 2006). Variable protein level in rotifiers in the present study may have been influenced by palm oil type that affects the ingestion rate of rotifer with 24 h of the enrichment process. The acceptability of rotifer might be due to the preference of available food as a consequence of the response of rotifers upon encountering the supplied feed (Wallace, 1980; Hotos, 2003). Evidently, the enriched diet did not significantly affect the whole-body proximate composition which suggested that Asian seabass larvae efficiently utilized the enriched rotifer to support survival and growth.

CONCLUSION

Palm oil in the form of CPO and RPO can be successfully used as a dietary lipid source in the enriched diets for rotifer and is able to support good survival and growth of Asian seabass larvae. Replacing 75% fish oil with RPO performed better than the other palm oil-based enriched diets and the control. A successful replacement of fish oil with palm oil in the enriched diets for rotifer will be able to reduce larval rearing production cost of Asian seabass. It is also a significant finding in larval nutrition research that can contribute to sparing the natural populations of forage fish from unsustainable exploitation. Rotifer represents a suitable live prey for enrichment with the ingredients that positively influence the nutritional requirements of the early larval stages of the fish widely used in aquaculture. Further research toward optimizing the alternative sources
TABLE 9 | Fatty acid composition (% of total fatty acids) of Asian seabass.

| Fatty acid (One-way ANOVA) | FO-100 | CPO50 | CPO75 | RPO50 | RPO75 |
|---------------------------|--------|-------|-------|-------|-------|
| C14:0                      | 0.9 ± 0.0^d | 0.9 ± 0.1^a | 0.7 ± 0.0^b | 0.8 ± 0.0^c | 0.7 ± 0.1^b |
| C15:0                      | 0.5 ± 0.0^ae | 0.5 ± 0.0^ab | 0.6 ± 0.0^a | 0.5 ± 0.0^a | 0.5 ± 0.0^c |
| C16:0                      | 26.5 ± 1.3^c | 32.2 ± 0.0^c | 33.3 ± 0.0^c | 30.8 ± 0.8^a | 32.4 ± 0.9^p |
| C16:1                      | 4.0 ± 0.0^a | 4.0 ± 0.1^a | 3.2 ± 0.2^a | 3.6 ± 0.1^b | 3.0 ± 0.0^c |
| C17:0                      | 1.9 ± 0.1^a | 1.7 ± 0.0^a | 1.7 ± 0.0^a | 1.4 ± 0.1^b | 1.4 ± 0.0^c |
| C18:0                      | 5.5 ± 0.1^a | 5.5 ± 1.0^a | 4.2 ± 0.4^c | 4.3 ± 0.4^c | nd |
| C18:1n9                    | 18.3 ± 0.0^c | 20.3 ± 0.0^b | 20.4 ± 0.1^b | 21.6 ± 0.6^c | 21.5 ± 0.6^p |
| C18:2n6                    | 1.0 ± 0.1^e | 0.9 ± 0.7^b | 0.2 ± 0.0^a | 0.2 ± 0.0^a | nd |
| C20:1                      | 0.6 ± 0.0^a | 0.7 ± 0.0^a | 0.8 ± 0.0^a | 0.7 ± 0.0^a | 0.7 ± 0.0^a |
| C20:4n6                    | 2.9 ± 0.0^a | 3.5 ± 0.0^a | 2.4 ± 0.0^a | 2.9 ± 0.1^c | 3.1 ± 0.1^b |
| C20:5n3                    | 3.9 ± 0.0^a | 4.3 ± 0.0^a | 2.6 ± 0.0^a | 3.9 ± 0.1^b | 3.8 ± 0.1^c |
| C22:6n3                    | 10.1 ± 0.3^a | 7.0 ± 0.3^b | 5.0 ± 0.8^cd | 4.6 ± 0.3^d | 6.0 ± 0.7^c |

2Fatty acid (One-way ANOVA) Parameters

| Parameters                  | Total saturates | Total monoenes | Total PUFA | n-3 | n-3/n-6 | DHA/EPA |
|-----------------------------|-----------------|----------------|------------|-----|--------|---------|
| **FO100**                   | 33.9 ± 1.2^a    | 23.5 ± 0.0^c   | 23.6 ± 0.4^b | 14.1 ± 0.3^a | 3.1 ± 0.0^a | 2.6 ± 0.1^c |
| **CPO50**                   | 40.8 ± 1.0^a    | 25.8 ± 0.0^a   | 17.6 ± 0.0^b | 11.4 ± 0.3^a | 2.1 ± 0.1^b | 1.6 ± 0.1^c |
| **CPO75**                   | 41.9 ± 1.0^a    | 25.0 ± 0.3^a   | 12.2 ± 0.7^a | 7.8 ± 0.8^b | 1.8 ± 0.2^b | 1.9 ± 0.3^c |
| **RPO50**                   | 37.7 ± 1.4^c    | 26.0 ± 0.6^a   | 14.9 ± 0.6^a | 8.7 ± 0.3^d | 1.4 ± 0.1^c | 1.2 ± 0.1^d |
| **RPO75**                   | 39.2 ± 1.5^c    | 25.9 ± 0.7^a   | 15.3 ± 0.5^c | 9.9 ± 0.6^c | 1.8 ± 0.1^b | 1.6 ± 0.2^b |

Two-way ANOVA (p-value)

| Parameters                  | Palm oil type | Palm oil level | Oil type x oil level |
|-----------------------------|---------------|----------------|---------------------|
| P values                    | 0.005         | 0.000          | 0.084               |
| P values                    | 0.000         | 0.000          | 0.000               |
| P values                    | 0.000         | 0.000          | 0.000               |

1Minor fatty acids (<0.2% from total fatty acids) were not included in the table.
Means with same superscripts in the same row and column are not significant differences between treatment (Duncan’s multiple range test, P > 0.05).

of protein and lipid from plants for live feeds will help in shaping the aquaculture industry consistent with the criteria of sustainable development.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was conducted following the Researcher’s guidelines on code of practice for the care and use of animals for scientific purposes (Universiti Malaysia Sabah).

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AUTHOR CONTRIBUTIONS

NS conducted the larval rearing experiment, raw data collection and analysis, and draft writing. RS leader of research project, designed the experimental layout, analysis of results, and manuscript writing. SM manuscript reviewing and improving presentation of data. FC involved in larval rearing design and execution. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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