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# The Use of Cyanobacteria *Arthrospira platensis* and Cladoceran *Daphnia magna* as Complementary Protein and Lipid Sources in Transitional Diet for Common Carp (*Cyprinus carpio* L.) Nursery

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## Abstract

**This study was conducted to evaluate the use of cyanobacteria *Arthrospira platensis* and cladoceran *Daphnia magna* biomass as complementary protein and lipid sources in diet supplementation for common carp (*Cyprinus carpio* L.) nursery. Three experimental diets containing *A. platensis* and *D. magna* meal at different concentrations were compared to the commercial (control) diet. Each experimental diet (ED) was set to contain *D. magna* and *A. platensis* meal at a specific combination: 2% and 5%, 2% and 7%, and 4% and 5% for ED1, ED2 and ED3, respectively. The protein and lipid content of the experimental diets ranged from 43.20% to 44.60% dry weight (DW) and 10.64% to 13.42% DW, respectively; while the protein and lipid content of the control diet were 43.00% DW and 6.72% DW, respectively. After 20 days of feeding period, ED3 group obtained the highest final body weight (BW) (58.18 ± 35.24 mg), total biomass (1936 ± 1625 mg), food conversion rate (1.34 ± 0.04), and specific growth rate (12.86 ± 0.03% BW day$^{-1}$) among all treatment groups ($P > 0.05$); while ED1 group obtained the highest survival (75.5% ± 7.47%) among all treatment groups ($P > 0.05$). The total bacterial count and total pathogenic *Aeromonas* sp. in the culture water of the treatment groups (ranged from 2.00 to 2.65 × 10$^5$ CFU mL$^{-1}$ and 3.50 to 4.12 × 10$^3$ CFU mL$^{-1}$, respectively) were lower compared to the water of the control group fed (3.73 × 10$^5$ and 4.70 × 10$^3$ CFU mL$^{-1}$, respectively). No significant differences in physicochemical water quality parameters were observed among treatments ($P > 0.05$). The current study suggests that the combination of**

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***A. platensis* and *D. magna* biomass can be used as complementary protein and lipid sources in diet supplementation for common carp larvae and can result in a comparable fish growth, survival and feed utilization in common carp culture at the nursery phase.**

## Keywords

***Arthrospira platensis*, Artificial Diet, *Cyprinus carpio* L., *Daphnia magna*, Nursery**

## 1. Introduction

Common carp (*Cyprinus carpio* L.) is one of the protein sources that are usually being served as special food in traditional or social event in Indonesia [1] [2]. In 2013, common carp production in Indonesia reached 340.863 metric ton, contributed 16.35% of the total production of freshwater fish in Indonesia [3]. Even though this commodity has become an important aspect in cultural and economic value, there was still a problem that became a bottleneck in common carp production, which is the availability of seed from larviculture and nursery stages that has to be brought into grow out pond [4]-[6].

In Indonesia, nearly all common carp nurseries are still conducted using traditional zootechnique, which involved external outdoor and earthen ponds, with less attention on water quality and lacking supply of live food. These conditions often lead to low survival in the nursery phase [5] [6]. Until now, the fulfillment of larviculture stage diet still entirely depends on live food. However, in nursery stage, as a transition stage from larviculture to grow-out, there are almost no appropriate diet available. Furthermore, this lack of diet has led to uncertainty in the fingerling production. There were several studies which showed that lack of live food for larvae had been considered as one of the main causes of low fish survival at the nursery phase [4] [7]. Another study showed that this high mortality could be reduced by 40% - 60%, by feeding the carp with a combined diet of live food and artificial diet [6]. Similar research also reported that the combination of cultured live prey organisms and dry diet in 1:1 diet formulation proportion resulted in the highest survival and growth of common carp [7]. These studies suggested that a transitional diet that partly uses live food as its constituent should be taken as a serious consideration in success of common carp nursery culture.

Live food offers numerous advantages, such as able to meet the most of seed nutrient requirements and highly digestible, as it contains enzyme systems which allow self autolyses in larval digestive system [8]. Live food *Arthrospira platensis* is a cyanobacterium that is rich in protein (60% - 70% in dry biomass) with a complete amino acid profile [9]. *Daphnia magna* is a cladoceran that is commonly used as live food in aquaculture production since it contains an autolysis enzyme system, as well as a high protein content (39.24% dry biomass) and amino acid profile that could meet the entire common carp requirements [10]. The aim of the current study was to evaluate the use of live feed *A. platensis* and *D. magna* biomass as complementary protein and lipid sources for transitional artificial diet supplementation for common carp **(***Cyprinus carpio* L.**)** culture at the nursery stage.

## 2. Materials and Method

### 2.1. Live Feed *Arthrospira platensis* and Daphnia Magna Biomass

#### 2.1.1. *Cyanobacteria Arthrospira platensis* Biomass Production

In each production cycle, 10% (*v/v*) of microalgae *Arthrospira platensis* stock culture was inoculated into a batch photobioreactor system at the density of $10^3$ cell $mL^{-1}$ and incubated for 10 days [11]. The photobioreactor system consisted of three major compartments: (1) 50 of 10 L algal rearing flasks containing Zarrouk medium ($NaHCO_3$ 16.8 g $L^{-1}$, $NaNO_3$ 2.5 g $L^{-1}$, $K_2HPO_4$ 0.5 g $L^{-1}$, $K_2SO_4$ 1 g $L^{-1}$, NaCl 1 g $L^{-1}$, $CaCl_2$ 0.04 g $L^{-1}$, $Na_2EDTA$ 0.08 g $L^{-1}$, $MgSO_4$. 7 $H_2O$ 0.2 g $L^{-1}$, $FeSO_4$. 7 $H_2O$ 0.01 g $L^{-1}$ [12], (2) fluorescence lamp providing continuous light at the intensity of 5000 ± 500 Lux, and (3) aeration system providing gentle aeration at the rate of 480 mL $minute^{-1}$. The water culture temperature was maintained at 25˚C ± 1˚C. Four production cycles were performed to obtain a total of 2,000 L *A. platensis* culture. After each production cycle, *A. platensis* biomass was harvested using a 110 µm nylon screen [12]. The harvested *A. platensis* wet biomass was dried in an oven at the

temperature of 60˚C and further milled into a powder form, further referred as *A. platensis* meal [13].

#### 2.1.2. Cladoceran Daphnia Magna Biomass Production

*Daphnia magna* was cultured in 2000 L rectangular tank (2 × 1 × 1 $m^3$). Culture conditioning was started with pond fertilization using 800 mg $L^{-1}$ urea and 15 mg $L^{-1}$ Triple Super Phosphate (TSP) to grow phytoplankton as the live feed for *D. magna*. Once the green water containing phytoplankton at $10^5$ cells $mL^{-1}$ was established, adult *D. magna* was inoculated at the density of 5 individuals $L^{-1}$ [8]. Each week 3 g of baker's yeast was added into the *D. magna* culture pond. Partial harvesting of *D. magna* was done every 3 days from week-2 onwards using a net with 700 µm mesh size. Harvested biomass were dried in an oven at the temperature of 60˚C. Dried biomass then milled into powder form, further referred as *D. magna* meal [13].

#### 2.1.3. Nutrient Composition of *A. platensis* and *D. magna* Biomass

Protein content of the produced *A. platensis* and *D. magna* meals was determined using Kjeldhal method, while their lipid content was determined using Soxlet method. Carbohydrate, ash and moisture content were determined by gravimetric method [14]. Additionally, the amino acid composition of *A. platensis* and *D. magna* biomass was determined using HPLC method [14], while the fatty acid composition was determined using gas chromatography of the fatty acid methyl esters (FAME) following the modified procedure by Greenfield and Southgate [14].

### 2.2. Experimental Diets

#### 2.2.1. Formulation of Experimental Diets

Beside *A. platensis* and *D. magna* meal, several diet raw materials were used as the ingredients of the experimental diets. Several meals such as fish meal, soy bean meal, shrimp waste meal, bone meal, wheat meal and premix aquavita were obtained from commercial animal feed manufacturer, CV Missouri, Bandung, Indonesia. Gelatin, dry yeast, fish oil, and soy lecithin were obtained from local cake store, PD Kijang Mas bakery supplies, Bandung. The nutrient content of the diet raw materials is presented in **Table 1**.

The ingredient composition of the experimental diets is presented in **Table 2**. Each of the experimental diets (ED) was set to contain *D. magna* and *A. platensis* meal at a specific combination: ED1 contained 2% and 5%, ED2 contained 2% and 7%, and ED3 contained 4% and 5%, respectively (**Table 2**). Based on the nutritional properties of the *D. magna* and *A. platensis* meal (**Table 3**), the three experimental diets were formulated to contain approximately 40% protein and 4.6% lipid content using Pearson square method [15] [19] [20].

#### 2.2.2. Preparation of Experimental Diets

The experimental diet preparation was done as follow: the dry feed ingredients were weighed and thoroughly mixed in a mixer before liquid and oily ingredients were added. The moist mixture was extruded through 1 cm screw stock and then crumbled into irregular shaped spheres using food processor form. The resulting moist pellets were air-dried in oven at the temperature of 60˚C to a moisture content of about 100 $g \cdot kg^{-1}$. Pellets were

**Table 1.** Nutrient composition of diet raw materials for experimental diets.

| Diet Ingredient | Protein (% DW) | Lipid (% DW) | Fiber (% DW) | Ash (% DW) | Moisture |
|---|---|---|---|---|---|
| Wheat Meal | 10.62 | 1.44 | 74.52 | 0.59 | 12.83 |
| Fish Meal | 38.70 | 8.58 | 0.15 | 44.42 | 8.83 |
| Soy Bean Meal | 45.77 | 1.88 | 35.97 | 6.41 | 9.97 |
| Bone Meal | 28.01 | 6.68 | 0.93 | 59.16 | 5.22 |
| Shrimp Waste Meal | 44.10 | 6.66 | 10.86 | 29.24 | 9.14 |
| Baker Yeast | 39.48 | 6.51 | 42.06 | 5.19 | 6.76 |
| Fish Oil | - | 100 | - | - | - |
| Soy Lecithin | NA | 69.41 | NA | NA | NA |
| Gelatin | 88.16 | 0.08 | 0 | 0.44 | 11.32 |

NA: Not available data.

**Table 2.** Formulation of the experimental diets.

| No | Ingredients | g·kg$^{-1}$ diet | | |
|---|---|---|---|---|
| | | ED1 | ED2 | ED3 |
| 1 | Fish meal | 400 | 350 | 311.4 |
| 2 | Soy bean meal | 230 | 234.5 | 265 |
| 3 | Gelatin | 100 | 100 | 100 |
| 4 | Shrimp waste meal | 80 | 87.3 | 51.1 |
| 5 | Fish Oil | 36.2 | 35.8 | 37.3 |
| 6 | Bone Meal | 12.3 | 30.9 | 73.7 |
| 7 | Wheat Meal | 30 | 30 | 30 |
| 8 | Soy Lecithine | 30 | 30 | 30 |
| 9 | Dry Yeast | 10 | 10 | 10 |
| 10 | Premix Aquavita | 1.5 | 1.5 | 1.5 |
| 11 | *A. platensis* meal | 20 | 20 | 40 |
| 12 | *D. magna* meal | 50 | 70 | 50 |

**Table 3.** Proximate analyses of *A. platensis* and *D. magna* biomass.

| Nutritional Component | *A. platensis* (%) | *D. magna* (%) |
|---|---|---|
| Protein | 69.00 | 44.47 |
| Lipid | 0.80 | 8.98 |
| Carbohydrate | 9.96 | 15.82 |
| Moisture | 11.63 | 11.59 |
| Ash | 8.61 | 19.34 |

ground into small pieces and sieved through 125 µm opening mess to produce pellet that is suitable with common carp mouth size [16]. The proximate composition of experimental diets including protein content was determined using Kjeldhal method, lipid content was determined using Soxlet method, then carbohydrate, ash, and moisture were determined using gravimetric method [14]. Furthermore, the essential amino acid and essential fatty acid content of experimental diet were determined using HPLC method and chromatography of the fatty acid methyl esters (FAME) following the modified procedure by Greenfield and Southgate [14].

## 2.3. Experimental Setup

Four day old Common carp larvae (*Cyprinus carpio* L.) were obtained from commercial hatchery in Cianjur, West Java, Indonesia. Upon collection, fish were acclimated in a 100 L aquarium for 10 days prior to stocking in the experimental units. At the end of the acclimatization period, fish larvae were weighed and randomly stocked into 12 of 10 L (0.3 × 0.2 × 0.2 m$^3$) aquaria at the density of 100 larvae per aquarium. Zero-water discharge (ZWD) system containing 0.5 L of nitrifying bacteria consortium at the density $10^7$ CFU mL$^{-1}$ supplied with $CaCO_3$ powder was prepared to maintain a low ammonium and nitrite level in each experimental system Southgate [11]. Water temperature was maintained at 28˚C ± 1˚C using electric water heater and continuous gentle aeration was provided through air diffuser tubing inside each system at the rate of 480 mL minute$^{-1}$. The fish larvae were fed at a fixed daily feeding level of 10% body weight in five equal meals daily (at 08.00, 11.00, 15.00, 18.00, and 20.00) during the 20 days of experimental period. Approximately 5% of total water volume in each aquarium was siphoned and replaced with new water every 4 days to remove remaining uneaten diet and fish feces. All experimental diets were evaluated in 3 replicates.

## 2.4. Water Quality Parameters

The physicochemical water quality parameters, including temperature, pH level, and dissolved oxygen (DO),

ammonium, nitrite, and nitrate concentrations were measured every four days during the experimental period. Dissolved oxygen and water culture temperature were measured using DO meter portable (Hach, USA) and pH level was determined using portable pH meter (Hanna Instrument, USA). Ammonium, nitrite and nitrate concentration was measured using spectrophotometric method at the wavelengths ($\lambda$) of 420 nm (Nessler method), 520 nm (Diazotization method), 275 and 220 nm (Nitrate-HCl method), respectively [17].

The microbiological water quality parameters, including the total heterotrophic bacteria (Total Heterotrophic Bacterial Count) and total number of *Aeromonas* sp. of the culture water were performed at the beginning (day 0), middle (day 10), and end of culture period (day 20). 1 mL of culture water was sampled from each rearing tank were diluted ($10^{-1}$ to $10^{-7}$) using sterile physiological solution (0.85% NaCl). From the dilutions, 0.1 ml was spread in duplicate on Nutrient Agar plates (Oxoid, USA) to determine the total bacterial count, as well as on Ryan medium agar plates to determine the total number of *Aeromonas* sp. (Oxoid, USA). The inoculated plates were incubated at 25˚C and bacterial counts were performed following 24 to 72 h incubation period [18].

### 2.5. Growth and Survival Parameters

Fish growth (average individual body weight and length), survival, food conversion ratio (FCR), and total biomass were measured at the beginning (day 0) and at the end (day 20) of the culture period. Measurements were conducted on all stocked and harvested juveniles. Fish specific growth rate was calculated by using the following equation [11]:

$$\mathrm{SGR}\left(\%\,\text{weight increase per day}\right)=\left[\frac{\left(\mathrm{InW}-\mathrm{InW}_0\right)}{\mathrm{t}}\right]\times 100$$

where W is the average body weight after 20 days, $W_0$ is the average initial body weight, and t is experimental period (20 days). The same approach was used to calculate the feed conversion ratio (FCR), expressed as the feed consumption over the weight increase of the fish per treatment. Fish survival for individual treatments was determined as the number of surviving fish at the end of experimental period relative to the number of fish at the beginning of the experimental period.

### 2.6. Statistical Analyses

Prior analyses, normalization of the distribution of the survival and individual final body weight data were done using arcsine and log transformation, respectively. Comparison of the fish survival, final BW and length, SGR and FCR were done using one-way analysis of variance (ANOVA). Grouping of treatments based on significant differences in mean values was done using a Tukey's multiple range post-hoc test (0.05 level of confidence). STATISTICA statistical software (version 7.0) was used for all statistical analyses.

## 3. Results

### 3.1. *A. platensis* and *D. magna* Biomass Production and Nutrient Profile

The productivity of the *A. platensis* culture within 10 days cultivation period was 0.259 g $L^{-1}$ (±10% of wet weight biomass) while the productivity of the *D. magna* culture within 5 days cultivation period was 0.04 g $L^{-1}$ (±8% of wet weight biomass). The proximate analysis of the *A. platensis* and *D. magna* biomass is presented in **Table 3**. In this study, the produced *A. platensis* biomass was found to contain 69% protein, 0.80% lipid, 9.96% carbohydrate, 8.61% ash and 11.63% moisture; while the *D. magna* biomass contained 44.47% protein, 8.98% lipid, carbohydrate 15.82%, moisture 11.59%, and ash 19.34%.

**Table 4** presented the essential amino acids profile of the *A. platensis* and *D. magna* biomass. In general, the *A. platensis* biomass was found to contain higher level of essential amino acids compared to *D. magna* biomass (**Table 4**).

The essential fatty acids profile of *A. platensis* and *D. magna* biomass are presented in **Table 5**. It was shown that *A. platensis* had lower essential fatty acid and did not contain linolenic acid, docosahexaenoic acid (DHA), and arachidonic acid (ARA). In contrast with *A. platensis*, *D magna* contained high level of essential fatty acids, particularly n-6 (**Table 5**).

**Table 4.** Essential amino acid profile of *A. platensis* and *D. magna* biomass.

| Parameter | % dry weight | | |
|---|---|---|---|
| | *A. platensis* | *D. magna* | Diet Requirement [19] |
| Histidine | 1.03 | 0.89 | 0.8 |
| Arginine | 4.23 | 2.25 | 1.6 |
| Threonine | 2.95 | 2.23 | 1.5 |
| Valine | 3.52 | 2.27 | 1.4 |
| Methionine | 1.20 | 0.98 | 0.8 |
| Lysine | 2.85 | 2.09 | 2.2 |
| Isoleucine | 3.19 | 1.86 | 0.9 |
| Leucine | 5.08 | 3.03 | 1.3 |
| Phenylalanine | 2.72 | 2.29 | 1.4 |

**Table 5.** Essential fatty acid profile of *A. platensis* and *D. magna* biomass.

| Parameter | % dry weight | |
|---|---|---|
| | *A. platensis* | *D. magna* |
| Linoleic Acid | 0.13 | 0.33 |
| Linolenic Acid | 0 | 0.15 |
| ARA | 0 | 0.04 |
| EPA | 0.01 | 0.03 |
| DHA | 0 | 0.03 |
| Omega-6 | 0.13 | 0.37 |
| Omega-3 | 0.01 | 0.21 |

### 3.2. Nutrient Composition of Experimental Diets

Proximate analysis of the experimental diets is presented in **Table 6**. The experimental diets contained the necessary nutrient level required by the fish larvae [19] [20]. Essential amino acids composition of the experimental diets is presented in **Table 7**, and the essential fatty acids composition is presented in **Table 8**. The control diet had the highest total essential amino acid content among experimental diets. It is shown that ED1 had the highest total essential amino acid content among experimental diets (**Table 7**), while ED3 has the highest linoleic acid and total n-6 content and ED2 has the highest DHA and total n-3 content among experimental diets (**Table 8**).

### 3.3. Water Quality Parameters

There were no significant differences in the physicochemical water quality parameters among treatments ($P > 0.05$). During 20 days of experimental period, the water temperature, dissolved oxygen and pH was stable, ranged between 29˚C to 31˚C, 5.1 to 6.2 mg $L^{-1}$, and 8.1 to 8.4, respectively. The ammonium ( $NH_4^+ - N$ ) and nitrite ( $NO_2^- - N$ ) concentration ranged between 0.6 - 1.8 mg $L^{-1}$ and 0.3 - 3.3 mg $L^{-1}$, respectively.

In terms of microbiological water quality parameters, there were no significant differences in total heterotrophic bacteria and total *Aeromonas* count in the culture water among treatments ($P > 0.05$). In all treatment groups, the ranges for total heterotrophic bacteria and total *Aeromonas* count in the culture water were between $10^5$ to $10^6$ CFU $mL^{-1}$ and $10^3$ to $10^4$ CFU $mL^{-1}$, respectively (**Table 9**).

### 3.4. Growth and Survival Parameters

After 20 days of experimental period, specific growth rate (SGR) of control group was considerably higher compared to ED1 and ED2 group but was not significantly different with ED3 group ($P > 0.05$). The highest SGR 13.18% ± 0.38% BW $day^{-1}$ with the lowest FCR 1.19 ± 0.08 were obtained by the control group ($P > 0.05$).

**Table 6.** Proximate analyses of the experimental diets compared to control feed.

| Nutritional Component | Larvae Requirement | Diet | | | |
|---|---|---|---|---|---|
| | | Control | ED1 | ED2 | ED3 |
| Energy (kJ/g) | 13 - 15 [19] [20] | 14.54 | 14.36 | 14.41 | 13.82 |
| Protein (%) | 43 - 47 [19] [20] | 43.00 | 43.77 | 44.62 | 43.18 |
| Lipid (%) | >4.6 [19] [20] | 6.72 | 13.42 | 12.49 | 10.64 |
| Fiber (%) | <38.5 [20] | 34.16 | 11.82 | 13.39 | 15.50 |

**Table 7.** Essential amino acid composition of the experimental diets.

| Amino Acid | Diet (% dry weight) | | | |
|---|---|---|---|---|
| | Control | ED1 | ED2 | ED3 |
| Histidine | 1.32 | 1.15 | 0.75 | 0.94 |
| Arginine | 2.46 | 2.67 | 2.55 | 2.87 |
| Threonine | 1.53 | 1.53 | 1.5 | 1.52 |
| Valine | 1.77 | 1.91 | 1.88 | 1.78 |
| Methionine | 0.59 | 0.61 | 0.67 | 0.56 |
| Lysine | 2.30 | 2.65 | 2.56 | 2.05 |
| Isoleucine | 1.47 | 1.33 | 1.39 | 1.45 |
| Leucine | 2.45 | 2.23 | 2.26 | 2.36 |
| Phenylalanine | 1.80 | 1.47 | 1.49 | 1.75 |
| Total | 15.69 | 15.55 | 15.05 | 15.28 |

**Table 8.** Essential fatty acid composition of the experimental diets.

| Fatty Acid | Diet (% dry weight) | | | |
|---|---|---|---|---|
| | Control | ED1 | ED2 | ED3 |
| Linolenic Acid | 0.01 | 0.00 | 0.01 | 0.01 |
| Linoleic Acid | 0.42 | 0.38 | 0.32 | 0.67 |
| ARA | 0.08 | 0.11 | 0.10 | 0.10 |
| EPA | 0.01 | 0.01 | 0.01 | 0.01 |
| DHA | 0.01 | 0.01 | 0.02 | 0.01 |
| Omega 6 | 0.50 | 0.49 | 0.42 | 0.77 |
| Omega 3 | 0.03 | 0.02 | 0.04 | 0.02 |

**Table 9.** Total bacteria count during culture period.

| Treatment | Total Bacteria Count (CFU $mL^{-1}$) | |
|---|---|---|
| | Heterotrophic | *Aeromonas* sp. |
| Control | $3.73 \times 10^5$ | $4.70 \times 10^3$ |
| ED1 | $2.65 \times 10^5$ | $3.53 \times 10^3$ |
| ED2 | $2.76 \times 10^5$ | $4.12 \times 10^3$ |
| ED3 | $2.00 \times 10^5$ | $3.50 \times 10^3$ |

There were no significant difference on the fish survival and final total biomass among treatments ($P > 0.05$) (**Table 10**). ED2 group had the highest survival of 75.5%, followed by ED1 group 67.5%, ED3 56.0% and control group with the lowest survival of 53.0% ($P > 0.05$). The highest total biomass was obtained in control group with 3424 ± 1181 mg and the lowest total biomass was in ED2 treatment (1615 ± 292 mg) ($P > 0.05$).

**Table 10.** Growth and survival parameters (Mean ± St. Dev) following 20 days of experimental period.

| Parameter | Diet | | | |
|---|---|---|---|---|
| | Control | ED1 | ED2 | ED3 |
| Final body length (cm) | 1.64 ± 0.65[a] | 1.46 ± 0.38[b] | 1.44 ± 0.38[bc] | 1.55 ± 0.34[ab] |
| Final body weight (mg) | 73.98 ± 35.43[a] | 47.86 ± 23.63[a] | 57.40 ± 10.38[a] | 58.18 ± 35.24[a] |
| Total biomass (mg) | 3424 ± 1181[a] | 1704 ± 1258[a] | 1615 ± 292[a] | 1936 ± 1625[a] |
| Survival (%) | 53.0 ± 3.8[a] | 67.5 ± 4.2[a] | 75.5 ± 7.5[a] | 56.0 ± 4.0[a] |
| FCR | 1.19 ± 0.08[a] | 1.62 ± 0.10[a] | 1.37 ± 0.19[a] | 1.34 ± 0.04[a] |
| SGR (% BW day$^{-1}$) | 13.18 ± 0.38[a] | 11.60 ± 0.02[b] | 11.13 ± 0.52[b] | 12.86 ± 0.03[a] |

Different superscript letters within a column denote significant differences ($P < 0.05$).

## 4. Discussion

### 4.1. Nutrient Composition of *A. platensis* and *D. magna* Biomass

Similar to the study by Belay [9], the live feed cyanobacteria *A. platensis* biomass produced in this study contained a high level of protein, ranged between 50% to 70% dry weight. The other live feed, cladoceran *D. magna* biomass, was also found to contain a relatively high protein and lipid content (44.47% and 8.98%, respectively). This was higher when compared to the study by Boguti *et al*. [10] that reported a protein and lipid content of *D. magna* of 40% and 5%, respectively. The difference in the nutritional (protein and lipid) content of *D. magna* biomass in this study compared to the reference might be due to the different food sources given to the *D. magna* culture [8]. In this study, *D. magna* was fed with phytoplankton and baker's yeast, which may increase the lipid and protein content of *Daphnia* culture [21].

In general, *A. platensis* biomass was found to contain higher level of essential amino acids compared to *D. magna* biomass (**Table 4**). Nevertheless, the essential amino acid composition of both *A. platensis* and *D. magna* biomass fit to the essential amino acid composition required for common carp larvae [19] [20]. Unlike its essential amino acids composition, the *A. platensis* biomass had lower level of essential fatty acids compared to the *D. magna* and did not contain linolenic acid, docosahexaenoic acid (DHA), or arachidonic acid (ARA). The produced *D. magna* biomass contained high level of essential fatty acids, particularly omega 6, although the quantity was still below optimum for common carp larvae requirements (1% for linolenic acid, 1.1% for omega 3, and 1.1% for omega 6) [22]. The comparative advantage between *A. platensis* and *D. magna* on amino acid and fatty acid profile would thus become an ideal complement in the formulation of artificial transitional diet for common carp larvae culture.

### 4.2. Nutrient Composition of the Experimental Diets

Based on the results of proximate analyses of the experimental diets, each of the experimental diets had met the larvae nutrient requirements, including >40% of crude protein and >4.6% lipid content [19] [20]. All three experimental diets tested in this current study contained higher lipid content than the control diet to compensate for the energy requirement (energy >13 kJ g$^{-1}$; [20]) due to a lower fiber content (**Table 6**).

In general, each of the experimental diets has a complete essential amino acid profile and met the larvae requirements as recommended by Hasan [19]. It was suggested that essential amino acids play various functional properties, such as catalyst, structural, movement, regulation, genetic expression, immunity and support growth [23]. In terms of quantity, the control diet had the highest total essential amino acid content among each treatment. ED1 had the highest total essential amino acid content among experimental diets; despite that similar to the control diet, it cannot fulfill larval methionine optimum requirement [19]. ED3 cannot fulfill the larval methionine and lysine optimum requirement, whereas ED2 cannot fulfill the larval histidine and methionine optimum requirement [19]. In general, unlike the essential amino acids, the experimental diets tested in the current study contained essential fatty acids but at the level below the common carp larvae optimal requirements, *i.e*. 1% for linoleic and linolenic acid, 0.05% for n-3, and 1% for n-6 [20].

### 4.3. Water Quality Parameters

There were no significant differences in water physicochemical parameters, including the water temperature,

dissolved oxygen and pH level, and ammonium and nitrite concentration, among treatment groups. The water pH of each treatment was relatively alkaline because of $CaCO_3$ application for nitrifying bacteria substrate in zero water discharge system [11]. Overall, these water physicochemical parameters range were within acceptable limit for common carp larvae production [24].

As expected, the microbiological parameters of the culture water during the experimental period were within acceptable limit in aquatic environment, which ranged between $10^5$ to $10^6$ for total bacterial count and $10^3$ to $10^4$ for total *Aeromonas* count [25]. The results indicated that the risk of infection by either opportunistic or pathogenic bacteria was relatively low. This suggested that the use of the experimental diets for common carp nursery in ZWD system was able to control the bacterial population of the culture.

### 4.4. Growth and Survival Parameters

In this study, the higher specific growth rate (SGR) ($P < 0.05$) and lower food conversion rate (FCR) ($P > 0.05$) obtained by the control diet might be caused by the high energy and total essential amino acid content in control diet. The control diet also had the highest levels of essential amino acid content that may support larvae growth performance, such as leucine, isoleucine, and phenylalanine. Leucine and isoleucine may help stimulate fish growth and reduce feed conversion [26], whereas phenylalanine content in diet can increase feed efficiency and produces tyroxine (T4), which increase the growth performance [27]. Experimental diet ED3 that has no significant difference on SGR compared to control group was found to contain high essential fatty acids that may support growth such as leucine, isoleucine and phenylalanine.

The highest survival in ED2 treatment group ($P > 0.05$) may due the essential amino acids and essential fatty acids content in the ED2 diet. Notwithstanding the fact that total essential amino acid in ED2 was lower than control diet and ED1, ED2 has the highest methionine and the lysine content among diets. Methionine is one of the most essential amino acid that becomes a limiting factor in diet [28]. Methionine deficiency in diet can inhibit transcription process, which inhibits some enzyme formation [29]. The high content of methionine in ED2 is thought to be the reason for the high survival during culture period, considering that methionine has many roles related with regulation and metabolic pathway mediating [29]. Beside methionine, lysine often becomes one of the most limiting amino acid in commercial fish diet [28]. Lysine deficiency in diet can contribute to increasing mortality and lordosis probability in larva [23]. One of lysine's significant roles that may affect the survival of larvae is it could serve as substrate for carnitine synthesis, which is vital in larval protection from ammonia toxicity and other environmental factors [30].

The high content of essential fatty acid omega 3 also becomes important factor that contribute to survival of larvae in ED2. Although the essential fatty acid omega 6 content in ED2 was lower than the other diets, the highest activity of essential fatty acid of freshwater fish comes from omega 3. This correlates with the higher content of omega 3 than omega 6 deposition in freshwater fish tissue [20]. High activity of essential fatty acid can also decrease the probability of the fish to become essential fatty acid deficient. The symptom of essential fatty acid deficiency was not seen in direct way, but it causes poor growth and low survival of larvae [20].

## 5. Conclusion

In conclusion, the findings of this study suggest that the combination of live feed cyanobacteria *Arthrospira platensis* and cladoceran *Daphnia magna* biomass can be used as an alternative of protein and lipid sources. The current study also suggests that the different experimental diets supplemented with combined *A. platensis* and *D. magna* meal at different concentrations can result in a comparable fish growth, and thus they can be potentially used on common carp culture at the nursery stage. Further research needs to focus on the optimization of the combination of the live feeds, as well as the optimum experimental diet formulation, in order to obtain a better protein and lipid profile that will allow higher growth and/or survival of the fish larvae culture.

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