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Original Article

Evaluation of the population growth and fatty acid composition of Copepoda, *Oithona nana*, fed on different diets

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Abstract: The marine Copepoda species *Oithona nana*, is considered as one of the most Copepoda species that successfully mass cultured in marine hatcheries. This study investigated the effects of four feed diets (soybean, yeast, rice bran, and corn starch) on the population growth, growth rate, population composition, fecundity, and fatty acid composition of Copepoda, *O. nana*. The experiment was continued for 15 days and the copepods were fed on four feed diets with concentration of 1 g/10^6 individual/day. The results found that *O. nana* fed on corn starch showed the highest significant population growth (9067 Individual/L) and population growth rate (0.735). For nutritional value, copepods fed on rice bran were detected to have the highest content of monounsaturated fatty acid (MUFA), polyunsaturated fatty acids (PUFA), the lowest saturated fatty acids/unsaturated fatty acids ratio (SFA/UFA ratio) and the lowest SFA. More importantly, the rice bran diet was the only diet that showed eicosapentaenoic acid (EPA; C20:5ω3). Moreover, copepods fed on rice bran showed the highest significant female fecundity (8.33 egg/female), copepodite and nauplii percentages (33.27 and 32.65%, respectively). Finally, regarding to the quantity, corn starch is the most suitable diet for mass culturing *O. nana,* while, regarding to the quality, rice bran enhances the nutritional value and fecundity of the Calanoida Copepoda *O. nana.*

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Introduction

Livefoods are considered as one of the most key components of the production of marine fish and shrimp larvae in the marine hatcheries, still one of the most significant obstacles for the development of marine larvae production (Abdel Rahman et al., 2008, 2010; Khairy and El-Sayed, 2012; Ashour and Kamel, 2017). Livefoods are divided into two types of micro-organisms; phytoplankton (microalgae) and zooplankton (*like* rotifer, artemia, and copepods). Microalgae are the basic food chain in aquatic environments (Ashour 2015, 2020). Sequentially, livefoods (phyto and/or zooplankton), are the main live-foods that consumed by marine fish and shrimp larvae. In general, marine algal-cells have many bioactive compounds *like* PUFA, essential amino acids, carotene, flavonoid, and phenolic compounds, showing antimicrobial and antioxidant activities, non-specific immunity, and growth promoting activities (Hassan et al., 2017, Ashour et al., 2020a, 2020b, Elshobary et al., 2020). Many livefoods species meet the complete nutritional requirements of marine fish and shrimp larvae, therefore, livefoods are considered “nutritional bags” for marine larvae and postlarvae (Drillet et al., 2011; Sharawy et al., 2020; Abo-Taleb et al., 2020a). The production of marine microalgae is more difficult than the production of marine zooplankton because of many consecrations. In marine hatcheries, despite the complexity of livefoods productions for marine larvae, there are many benefits and potential of consuming livefoods for aquatic animals (Drillet et al., 2011).

Copepoda are one of the most nutritious livefoods used in marine hatcheries. Despite rotifer and *Artemia* were extensively used as preys in marine hatcheries (Heneash et al., 2015; Ashour et al., 2019), copepod species are considered the best live preys, due to their higher nutritional value than rotifers and *Artemia*

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(Støttrup, 2000; Olivotto et al., 2010; Drillet et al., 2011; Abate et al., 2016; Øie et al., 2017). In wild, Copepoda acting as trophic linkages between primary producers and secondary consumers in marine ecosystems (Støttrup, 2000; Ashour et al., 2018; Abo-Taleb et al., 2020b). Among Copepoda, the order Calanoida is considered the main organic matter consumer and energy transporter to higher trophic levels, including small fish, larvae, and juveniles of aquatic species in the marine ecosystem. Thus, Calanoida represents a major prey source for mesopelagic and bathypelagic fish (Yamaguchi et al., 2015; Abo-Taleb et al., 2020b). Calanoida Copepoda, especially species *Oithona nana,* is an excellent source of highly polyunsaturated fatty acids which makes copepods to be more nutritious and attractive food for larval and small fish (Drillet et al., 2011).

Selecting a suitable feed diet for Copepoda is a crucial key factor that extensively influences the quantity and quality of cultured copepods (Kleppel et al., 2005; Van der Meeren et al., 2008; Pan et al., 2018). Microalgae are the basic live diet utilized in marine hatcheries for copepod cultures (Vidhya et al., 2014; El-khodary et al., 2020). Microalgal are more preferred for copepods due to many factors such as nutritional value, size, shape suitability, and digestibility (Pan et al., 2018). Many studies have focused on the evaluation of a convenient microalgal diet for Copepoda species (Payne and Rippingale, 2000; Milione and Zeng, 2007; Camus et al., 2009; Ohs et al., 2010; Pan et al., 2018). However, the high labor and high production cost of microalgae are increases the copepods price production. Subsequently, the seeking of an optimal diet as microalgal alternatives diet for Copepoda species is critical for sustainable marine aquaculture, in particular for cultured species that have commercial prospects.

Many feed regimes were referenced as microalgal-alternative diets for different cultured copepods and for zooplankton species, in general, such as baker's yeast *Saccharomyces cerevisiae* (Payne and Rippingale, 2000; Farhadian et al., 2008), fish diet (Ribeiro et al., 2011), soybean (Agbakimi et al., 2017; El-khodary et al., 2020), rice bran (Depauw et al., 1981; Mubarak et al., 2017; Amian et al., 2018), starch and albumen (Sulehria et al., 2010), and glucose (Gyllenberg and Lundqvist, 1978). According to references, the alternative feeding regimes for culturing either copepods or zooplankton species were resulted in adequate population growth and productivity, depending on cultured species, culture methods, and experimental conditions. Therefore, studying the effects of feed types on copepods quality and quantity is need to find out their ideal prospect in marine aquaculture.

To select the optimal food regime for Calanoida Copepoda, *Oithona nana*, the current study was conducted to evaluate the population growth, population growth rates, population compositions, fecundity, and fatty acid compositions of *O. nana* fed on different diets of the commercial-grades of soybean, yeast, rice bran, and corn starch.

Materials and Methods

Copepods stock culture: The marine copepods were isolated from an earthen pond at El-Max Research Station, Alexandria Branch of National Institute of Oceanography and Fisheries, (NIOF). During the copepod collections period in spring 2017, the earthen pond temperature (23±2°C), salinity (31±1 ppt), and pH (7.37±0.10) were recorded at noon. Copepod samples were collected following the protocol described by Abo-Taleb et al. (2020a). Isolated individuals were initially examined using a binocular stereomicroscope (Optika Microscopes, B190/B-290, Italy), at 10X magnification. Morphological identification and taxonomic characterization was conducted by the Hydrobiology Lab., Marine Environment Division, NIOF, using the key references of Bradford-Grieve (1994), Newell and Newell (1933), and Gonzalez and Bowman (1965). After morphological classification, the isolated adult copepods were identified as Copepoda Calanoida: *Oithona nana* (Fig. 1). Adult individuals of *O. nana* were cultured under laboratory-controlled conditions (27±1°C, 20 ppt, pH 7.7±0.15, and continuous gentle aeration) and enriched with microalga *Nanno-*

Figure 1. Isolated adult Copepoda (Calanoida: *Oithona nana*).

chloropsis oceanica NIOF15/001 (5×10^6 cells/ml).

Regime and experimental design: Commercial grades of soybean, yeast (*Saccharomyces cervicates*), rice bran, and corn starch were used as feed for copepods. Yeast *S. cervicates* and corn starch was supplied by Starch and Yeast Company, Egypt, while soybean and rice barn was supplied from Fish Feed Factory located in Alexandria, Egypt. The copepods were fed on four regimes (treatments), including soybean, yeast, rice bran, and corn starch, with concentration of 1 g/10^6 individual/24-hr of each (Heneash et al., 2015). To prepare the concentration of the diets (three replicates for each treatment) of soybean, yeast, rice bran, and corn starch, 1 g of a commercial baker yeast and corn starch and a very finely grounded commercial soybean and rice bran were dissolved separately in 100 ml hot freshwater, shaken vigorously, and then blended using a kitchen mixer until fresh instant emulsion was formulated to be used as feeding regimes for copepods. The experiment was continued for 15 days, according to Santhanam and Perumal (2012).

The density of copepods in each food regime was estimated as individual/ml and the needed concentration for each food regime was estimated depending on the previously accounted copepods individual/ml every three days (day-0, 3, 6, 9, 12, and 15). Before experiment, the copepods were harvested from the stock culture tank and transferred to the new culture water for a 24 h gut evacuation to avoid the effects of resident soybean and algal diet (Tseng et al., 2009; Pan et al., 2018). At the beginning of the experiment, the adult copepods (with average size of 625 µm) were cultured in glass tanks field with 30 L of 1 µm bag-filtered, chlorine-disinfected of diluted seawater (20 ppt) with initial stock density of approximately 1 individual/ml (about 1,000 ind./l) with sex ratio 1:1. The culture conditions during the experiment were kept under controlled conditions of salinity 20 ppt, temperature 27±1˚C (using a digital thermometer), pH 7.7±0.15, and photoperiod 12:12. Each tank was hand-fed three times per day (9.00 am, 12.00 pm and 3.00 pm), seven days a week. The tanks were conducted without water replacement and were supplied with gentile aeration to keep dissolved oxygen (DO) over 4 mg/l (measured using Oxymeter, China). Ammonia (NH_3) concentration (measured using digital multi-meter, Italy) was <0.45±0.05 mg/l in all treatments, showed no negative effects of food regimes additions.

Tested Parameters

Population growth, growth rate, composition, and fecundity: Every three days, 25 ml of culture water from every replicate of each diet was taken to estimate the population growth of copepods, which estimated as increase in number (ind./ml). Every 3 days, about one hundred individuals from each replicate were harvested, using a 38-µm mesh, and fixed with a 4% formalin solution to estimate the percentage of population composition and different developmental stages (nauplii, copepodite, male, and female) under a microscope (Optika Microscopes, B190/B-290, Italy). Twenty to thirty vigorous carrying-females from every replicate were sorted and placed on a Petri dish to examine the fecundity. The population growth rate (r) was calculated according to Yin et al. (2013), using the equation of $R = (\ln N_t - \ln N_0)/t$, where N_0 and N_t are the initial and final population densities, and t is the incubation time in days.

Fatty acid analysis: At the end of the experiment (after day-15), all copepods of each replicate were harvested and preserved at −80°C for fatty acid

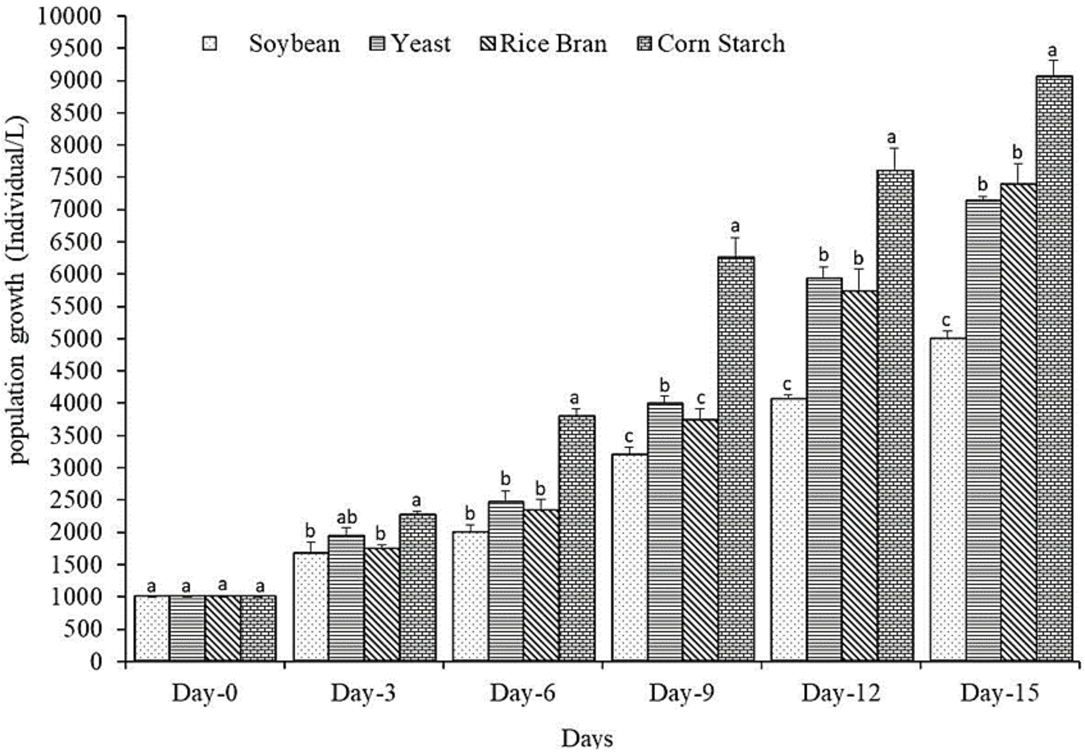

Figure 2. Effect of different diets on the population growth (individual/l) of Calanoida Copepoda *Oithona nana*. Data are presented as mean± standard errors. The letters (a, b, and c) above each bar indicate the significant differences ($P≤0.05$) between different diets in the same day.

analysis. Fatty acid profiles of *O. nana* fed different diets were extracted and estimated as described by El-Shenody et al. (2019).

Data analysis: Statistical analyses were analyzed using SPSS Version 16. The results are presented as the mean±standard error (n=3). All variables were evaluated in three replicates using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to compare differences among individual means at a significance level of $P≤0.05$.

Results

Population growth, growth rate, composition, and fecundity: Based on the results, the population growth (ind./ml), growth rate (r), population composition, and fecundity of *O. nana* were significantly affected by different diets (Figs. 2-5). Among all experimented diets, corn starch was exhibited the highest significant ($P≤0.05$) population growth and growth rate in all investigated days, including day-3 (2267 Ind./l and 0.273, respectively), day-6 (3800 Ind./l and 0.445, respectively), day-9 (6267 Ind./l and 0.611, respectively), day-12 (7600 Ind./l and 0.675, respectively), and day-15 (0967 Ind./l and 0.735, respectively), followed by yeast, rice bran, while the lowest population growth and growth rate was observed in soybean treatment (Figs. 2, 3). Figure 4 presents the percentages of the population composition (male, female, copepodite, and nauplii) of *O. nana* fed different diets showing *O. nana* fed on rice bran having the highest significant copepodite and nauplii percentages (33.27 and 32.65%, respectively) and the lowest significant male and female percentages (16.50 and 17.58%, respectively) (Fig. 4). Figure 5 shows the fecundity (eggs/female) of *O. nana* fed on different diets with *O. nana* fed on rice bran diet having the highest significant fecundity (8.32±0.167 eggs/female), followed by the corn starch (7.67±0.159 eggs/female), while the lowest one found in the soybean (6.17±0.177 eggs/female) and yeast (6.50±0.289 eggs/female).

Fatty acid Compositions: Fatty acid compositions were significantly varied in *O. nana* fed on the different diets (Table 1). Among all diets, *O. nana* fed rice bran showed the highest significant ($P≤0.05$) percentages of MUFA (34.87%) and PUFA (1.45%),

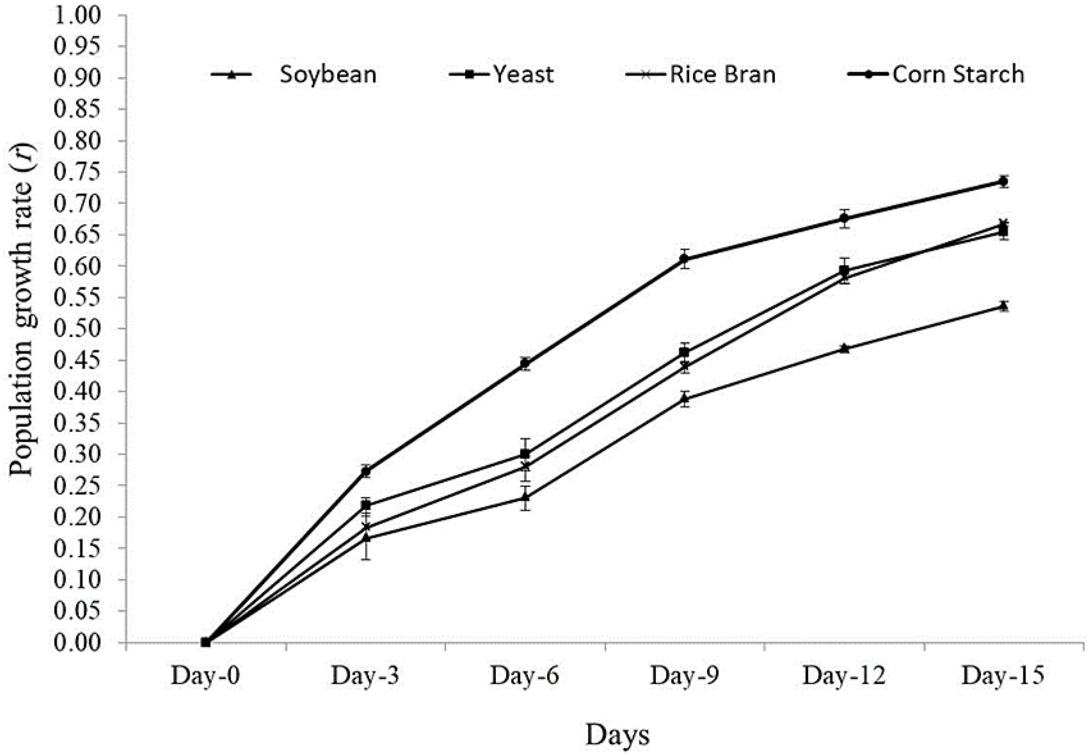

Figure 3. Effect of different diets on the population growth rate (r) of Calanoida Copepoda *Oithona nana*. Data are presented as mean±standard errors.

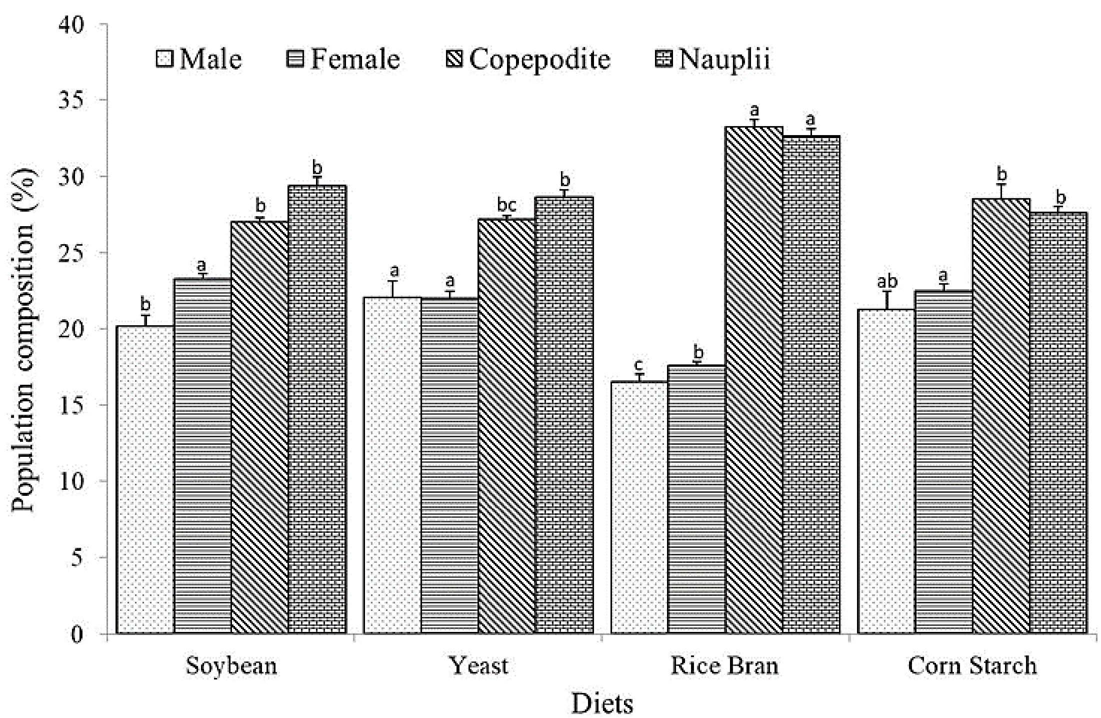

Figure 4. Effect of different diets on mean percentages of population compositions of *Oithona nana*. Data are presented as mean ± standard errors. The letters (a, ab, b, and c) above each bar indicate the significant differences ($P\leq0.05$) between the developmental stages (adult males, adult females, nauplii, and copepodites) in different diets.

and the lowest significant percentage of SFA (63.87%) and the lowest SFA/UFA ratio (1.77), compared to the others. Furthermore, *O. nana* fed on rice bran had a greater C18:1c (13.04%), C18:3ω3

Table 1. Fatty acid profiles (% of total fatty acids) of copepods fed on different food regimes.

FA	Soya Bean	Yeast	Rice Bran	Starch
SFA				
10:0	0.31±0.006^{d}	0.46±0.17^{a}	0.41±0.006^{b}	0.373±0.003^{c}
11:0	0.40±0.006^{c}	0.81±0.075^{a}	0.41±0.023^{c}	0.56±0.006^{b}
12:0	0.78±0.003^{ab}	0.88±0.058^{a}	0.70±0.020^{b}	0.52±0.035^{c}
13:0	2.17±0.040^{b}	2.87±0.055^{a}	2.39±0.205^{b}	3.10±0.159^{a}
14:0	10.51±0.309^{c}	17.80±0.820^{a}	14.15±0.471^{b}	16.10±0.393^{a}
15:0	0.91±0.147^{d}	2.60±0.101^{b}	1.99±0.012^{c}	11.80±0.115^{a}
16:0	38.54±0.12^{a}	24.78±0.303^{c}	30.48±0.029^{b}	24.83±0.245^{c}
17:0	0.55±0.003^{d}	1.92±0.043^{a}	1.16±0.015^{b}	0.69±0.003^{c}
18:0	21.88±0.245^{a}	11.57±0.205^{b}	9.76±0.064^{c}	8.12±0.090^{d}
20:4	0.83±0.046^{b}	2.65±0.191^{a}	2.43±0.300^{a}	2.22±0.049^{a}
MUFA				
14:1	12.35±0.001^{b}	15.16±0.823^{a}	15.58±0.003^{a}	13.93±0.543^{ab}
15:1	0.77±0.015^{b}	1.32±0.001^{b}	1.16±0.479^{b}	4.37±0.150^{a}
16:1	1.89±0.020^{b}	4.19±0.592^{a}	2.44±0.032^{b}	2.33±0.040^{b}
18:1c	6.22±0.150^{c}	9.07±0.297^{b}	13.04±0.069^{a}	9.02±0.433^{b}
18:2c	1.04±0.104^{c}	2.77±0.015^{a}	2.46±0.006^{b}	1.12±0.012^{c}
PUFA				
18:3ω3	0.83±0.038^{b}	1.18±0.017^{a}	1.20±0.061^{a}	0.91±0.012^{b}
20:5ω3	0.00±0.000^{b}	0.00±0.000^{b}	0.24±0.006^{a}	0.00±0.000^{b}
∑ SFA	76.89±0.297^{a}	66.33±0.479^{c}	63.87±0.647^{d}	68.32±0.084^{b}
∑ MUFA	22.27±0.260^{d}	32.49±0.514^{b}	34.68±5.95^{a}	30.77±0.095^{c}
∑ PUFA	0.83±0.037^{c}	1.18±0.017^{b}	1.45±0.055^{a}	0.91±0.012^{c}
SFA/UFA	3.33±0.058^{a}	1.97±0.043^{c}	1.77±0.052^{d}	2.16±0.009^{b}

Values are means±SE. Means (n = 3) in the same row with different superscript are significantly different (P≤0.05). FA: Fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; UFA: Unsaturated fatty acids.

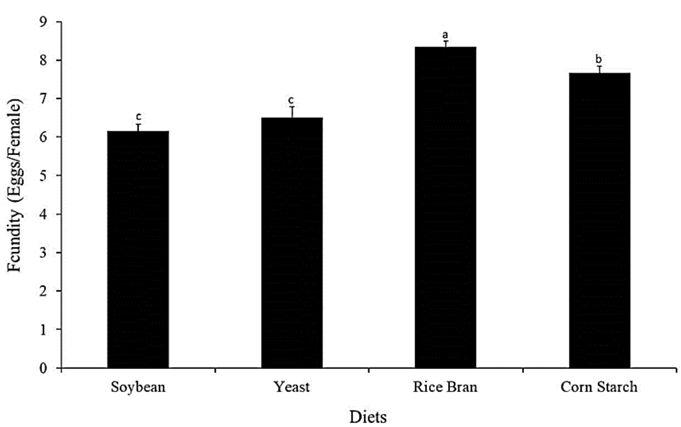

Figure 5. Effect of different diets on fecundity of *Oithona nana*. Data are presented as mean ± standard errors. The letters (a, b, and c) above each bar indicate the significant differences (P≤0.05) between different diets.

(1.20%). No recorded EPA (C20:5ω3) in all *O. nana* fed on the different diets expect the diet of rice bran which was the only that revealed a small amount of (0.24%) (Table 1).

Discussions

The low yields, long generation time, seasonal variations of production, and high costs are the main problems limiting the success of the culture of copepods (Støttrup, 2000; Conceição et al., 2010; Ajiboye et al., 2011). There are needs for cheap food to minimize the cost of productions copepod (Drillet et al., 2011). The commercial-grades of soybean, yeast, rice bran, and corn starch did not show any negative effects on the environment water quality during the experiment. Soybean meal is an attractive source of protein, which has high protein percentage, appealing smell, and low price (Booman and Jones, 2018). Moreover, it also contains different classes of specific anti-nutrients, including oligosaccharides and allergic proteins (FAO, 2011), while, the high concentration of soybean meal can be toxic (Barrows and Sealey, 2017). In marine hatcheries, Baker's yeast, *S. cerevisiae*, could be successively used as an algal-substitute, moreover, it has obvious benefits, like the reduction of algal production facilities and subsequently reduces the production cost. Many authors cited that the low concentration of yeast did not influence water quality (Payne and Rippingale, 2000; Farhadian et al., 2008; El-khodary et al., 2020). Rice bran was successfully used as feed for copepods, daphnia, *Artemia*, and moina cultures (Depauw et al., 1981; Mubarak et al., 2017). Amian et al. (2018) stated that the diversity and abundance Copepods, Rotifera, and Cladocera are enhanced when using rice bran in fish ponds during the rearing of tilapia. Rice bran, as well as soybean (Amian et al., 2018; El-khodary et al., 2020) must be processed into small particles suspension to fit the mouth of the cultured Copepods.

In current study, the highest significant population density and population growth rate were observed with copepods *O. nana* fed on corn starch diet. It may be due to its particle size, texture, energy content, and lower levels of anti-nutrients, or may be because of higher digestibility of corn starch. The result was in agreed with Sulehria et al., (2010), who cited that rotifers cultured on diet mixed with corn starch. The starch grains are composed of two types of alpha-glucan, amylopectin and amylose, which substitute approximately 98-99% of the dry weight of starch, moreover, starch contains relatively low quantities (0.4%) of minerals (Tester et al., 2004).

The findings of current study indicated that *O. nana* fed rice barn diet had a higher fecundity (8.33 eggs/female) compared with those fed on soybean, yeast, and corn starch (6.17, 6.50, and 7.67, respectively). Carli et al. (1995) reported that the type of diets (algae *Monochrisis lutheri* and yeast *Saccharomyces cerevisiae*) are strongly affects the fecundity and survival of harpacticoid Copepoda, *Tigriopus fulvus*. Previous studies have confirmed that the fecundity of copepods is probable to be linked to the content of PUFA in their diets (Støttrup and Jensen, 1990; Kleppel et al., 2005; Pan et al., 2018). The importance of dietary PUFA contents to the fecundity of *O. nana* was detected in the experimented diets.

In current study, the fatty acids composition of copepods fed on rice bran diet may explain the increases in female fecundity and comparing to the fatty acid compositions of copepods fed soybean, yeast, and corn starch, the fatty acid composition of copepods fed rice bran resulted in the highest significant MUFA and PUFA, as well as, the lowest significant SFA and the lowest SFA/UFA ratio. These funding may be due to the nutritional value of rice bran (Mubarak et al., 2017). Najeeb et al. (2015) cited that the commercial grade of rice bran contains 50% carbohydrate, 15% protein, 20% fatty acids (linoleic and oleic acids), 5% Vitamin E (tocatrienols, tocopherols, oryzanols, phytosterols), and a low amount of other micronutrients.

Many works cited that copepods have the ability to endogenously synthesize PUFA from short-chain fatty acids (Lee et al., 200; Monroig et al., 2013). Interestingly, in the current study, the copepods fed on rice bran are the only exhibited EPA in their fatty acids profile. This funding may be attributed to the nutritional value of rice bran. Moreover, the EPA contents of copepod fed on rice bran may be explained the high fecundity, as well as, the high significant percentage of nauplii (32.65%) and copepodite (33.27%) in population composition of *O. nana*. The trend observed on fecundity was similar to the examination of population composition that rice bran provided the highest total percentage of nauplii and copepodite population. Besides, the rice bran was markedly predominated by nauplii and copepodite, with relatively few adult males and/or female. Our finding is agreed with the results of Pan et al. (2018) who cited that the high PUFA content of the diet positively affecting the fecundity, nauplii, and copepodite population of Copepoda, *Apocyclops royi.*

Many dry-diets have been utilizing in copepods production, however, there ideal prospects on copepods (the quality and the quantity) are still needed. Overall, regarding to the high percentages of nauplii and copepodite, maximum fecundity, and improved fatty acid profiles, especially PUFA content of Copepoda, *O. nana*, the rice bran is the optimal diet for the culturing *O. nana*. While, regarding to improvement of the quantity of cultured *O. nana*, the corn starch diet is recommended to produce the maximum population density and the maximum growth rate.

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