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Nutritional value and production performance of the rotifer *Brachionus plicatilis* Müller, 1786 cultured with different feeds at commercial scale

Kamil Mert Eryalçın [1]

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Abstract

The rotifer *Brachionus plicatilis* is the first live feed in larviculture of marine fish species. Rotifer diets differ in their biochemical composition, physical properties, and production technology while feeding protocols largely vary among facilities. The objective of the present study was to determine the effects of two different forms of *Nannochloropsis oculata* and commonly used commercial diets on growth performance and biochemical composition of rotifers produced under commercial conditions. Rotifers were fed one of five different types of feed: Algome® (dried *Schizochytrium* sp.), Protein Plus® (PP), Inactive Baker's Yeast® (INBY), spray-dried *Nannochloropsis oculata* (SDN), or freshly cultured *Nannochloropsis oculata* (FN). Rotifers fed SDN diet resulted in significantly higher rotifer biomass during 16 days of semi-continuous culture, with an increasing biomass trend that lasted 11 days, high egg production, and egg-carrying female numbers, whereas rotifers fed PP showed highest ∑n-3, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid contents. Amino acid profiles of rotifers were enhanced by utilization of both INBY and SDN diets. Overall, the results indicated that SDN is optimal for long-term biomass production of rotifers. However, their nutritional profile needs to be enriched by feeding PP (EFA source) and INBY (EAA source) once desired biomass production is obtained.

Keywords Amino acids · Essential fatty acids · Growth · *Nannochloropsis oculata* · Rotifer · Spray-dried

Abbreviations

HUFA	Highly unsaturated fatty acids
PP	Protein Plus®
INBY	Inactive Baker's Yeast®
SDN	Spray-dried *Nannochloropsis oculata*

✉ Kamil Mert Eryalçın
eryalcin@istanbul.edu.tr

[1] Faculty of Aquatic Sciences, Aquaculture Department, Fish Nutrition & Phytoplankton-Zooplankton Culture Laboratory, Istanbul University, Ordu Cad., No. 8, Laleli, 34134 Istanbul, Turkey

FN	Fresh *Nannochloropsis oculata*
ARA	Arachidonic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
LA	Linoleic acid
LNA	Linolenic acid

Introduction

Rotifers *Brachionus plicatilis* and *Artemia* nauplii are the most common live prey used in commercial marine fish hatcheries, with their utilization mostly depending on larval mouth size (Rainuzzo et al. 1997; Reitan et al. 1997; Conceição et al. 2010; Hawkyard et al. 2016). Rotifers are still indispensable in larvae culture due to their suitable size and relatively simple production techniques. However, their nutritional quality and reliable production markedly depend on the type of feed used and the stable production of high-quality rotifers is crucial for commercial marine fish hatcheries. For instance, in comparison to copepods and natural preys for many marine fish larvae, rotifers have lower amounts of essential fatty acids, certain amino acids, vitamins, and minerals (Hamre et al. 2013). Commercial rotifer diets and enrichment products have different nutritional compositions, and this variation could affect the quality and quantity of produced rotifers and, eventually, that fact mostly has effect on fish larvae. Therefore, adequate feed and enrichment protocols are important to sustain the desirable nutritional properties of live feeds for marine fish larvae (Hamre et al. 2008; Matsunari et al. 2012).

Microalgae are still widely used in larval production as rotifer feed (Haas et al. 2016) or in green water production (Tendencia et al. 2015) in the form of either fresh (Patil et al. 2007), spray-dried (Harel et al. 2002), freeze-dried (Tibaldi et al. 2015), or paste (Schwarz et al. 2008). Several studies on antibacterial effects of microalgae show that microalgae addition in culture water of marine fish larvae decrease the incidence of bacterial diseases (Salvesen et al. 2000; Sharifah and Eguchi 2011) and play important role in fish larvae intestine, either directly or through rotifers (Ringø et al. 2014). Therefore, microalgae can be readily available in commercial hatcheries in different forms: fresh, paste, frozen, or freeze-dried. Fresh microalgae production expenditures in commercial marine fish hatcheries may count up to 30–40% of total production cost (Grima et al. 2003; Norsker et al. 2011). Therefore, many hatcheries are interested in the use of commercial microalgae products, despite their price may be elevated depending on their quality and purpose of utilization (Borowitzka 2013). Among them, fresh microalgae have beneficial antibacterial effect on larvae culture tank against pathogens such as *Vibrio* sp. (Shields and Lupatsch 2012; Taniguchi et al. 2011). Microalgae biomass can be also concentrated in paste form, which contains high cell density. However, unprocessed microalgae paste should be utilized within a couple of days in hatcheries, whereas freeze-dried microalgae forms have several advantages, such as easy utilization, maintained original cell shape, and texture with also preserved biochemical properties (Lubzens et al. 1995; Pedro and Fernandez-Diaz 2001). On the other hand, spray-dried microalgae can also be an alternative biomass. However, within this process, microalgae cells may become smaller and lose product quality (Ryckebosch et al. 2011).

Several commercial rotifer feeds such as concentrated suspensions, frozen biomass, micro-encapsulated, and yeast-based diets are available and their effect on rotifer production may differ (Srivastava et al. 2006; Dhert et al. 2014; Hamre 2016). This variation is mainly

dependent on the nutritional value and physicochemical properties of rotifer feeds and rotifer culture conditions and protocols. For instance, microcapsulated diets contain certain amount of vitamins, minerals, and highly unsaturated fatty acids (HUFA). For example, Culture Selco® has an optimum HUFA composition in comparison to most commonly utilized rotifer live feeds such as microalgae or baker's yeast. Besides, biochemical properties of paste form of microalgae mainly depend on cultured microalgae species and culture conditions. Therefore, it is more difficult to sustain certain amount of nutrients by fresh microalgae or paste (Dhert et al. 2001).

Cultivation type of rotifer is one of the factors that influence the rotifer culture success. Batch, semi-continuous, and high-density culture methods are applied depending on the needs of facilities, tank volumes, and feeds utilized in rotifer production. In batch culture, harvested rotifers are divided in two parts: new inoculation for rotifer and feeds for cultured species. In this method, high amounts of commercial feeds are given into the culture tank in short time, 3–4-day culture period. Partly harvesting and washing of rotifer cultures is known as semi-continuous culture. Compared to the batch culture method, semi-continuous culture can be longer due to periodically harvesting. High-density culture is also performed by the utilization of concentrated microalgae biomass. However, water quality and stability are the main problems in this method. In order to eliminate those problems, protein skimmers, filtering, and partly renewing water techniques could be applied in high-density culture methods (Yoshimatsu and Hossain 2014).

Nannochloropsis oculata has high phototrophic growth potential (Spolaore et al. 2006; Hemaiswarya et al. 2011) and is also rich in eicosapentaenoic acid (20:5n-3) and arachidonic acid (20:4n-6) essential fatty acids for marine fish larvae (Izquierdo and Koven 2011). Therefore, different forms of this microalgae species were prepared and investigated for rotifer culture. The aim of this study was to compare the performances of different forms of *Nannochloropsis oculata* and different commercial rotifers feeds in semi-continuous culture of rotifers during 16 days.

Materials and methods

Microalgae culture

Nannochloropsis oculata (The Culture Collection Algae and Protozoa (Strain number: CCAP 849/1), Scotland, UK) was cultured in f/2 medium (Guillard and Ryther 1962) previously sterilized at 121 °C for 15 min. All sub-cultures were maintained at 27 °C and salinity of 32 PSU under a 12L:12D photoperiod. *N. oculata* culture volume was up-scaled from 50-mL test tubes to 250-mL Erlenmeyer flasks, followed by 1-L, 5-L, and 300-L culture containers in tubular photobioreactors, continuously. Illumination of tubular stand was 200 μmol/m^2/s at the surface of culture. The population growth was daily determined by cell counting using Neubauer chambers (Fig. 1).

Spray-dried microalgae production

N. oculata biomass was harvested (day 14) at early stationary phase and spray dried at 90 °C processing heat. From 70 L fresh *N. oculata* to 160 g dry cell biomass was maintained. The spray-dried *N. oculata* biomass was stored at − 80 °C after produced.

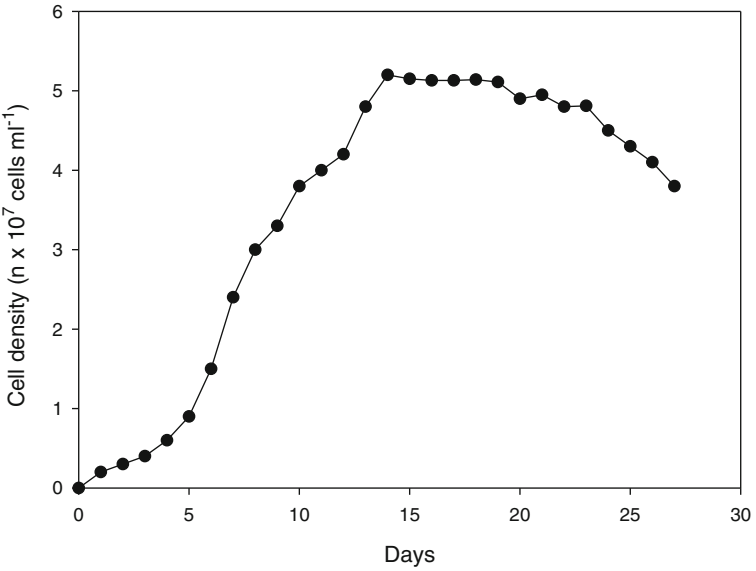

Fig. 1 Cell concentration of *N. oculata* during culture

Rotifer semi-continuous cultures

Prior to the beginning of the trial, rotifers (*Brachionus plicatilis*, L-type strain, lorica size 200–250 μm) were produced in 1000-L circular tanks with baker's yeast (*Saccharomyces cerevisiae*; Pakmaya, Turkey). Stock cultures were maintained in 1-L Erlenmeyer flasks with 30 rot/mL and cultivated in semi-continuously during culture period. Salinity (‰, 26), oxygen (6.5 ± 0.7 ppm), and temperature (26 ± 0.5 °C) were measured during experiment.

Experimental design and diets

This study was conducted in facilities of Akvatek Aquaculture Company, İzmir, Turkey. Initial rotifer stock density was 600–700 rot/mL in all experimental culture tanks. Rotifers were fed for 16 days semi-continuously with experimental diets. The rotifer feeds used were the following: Algome® (containing dried *Schizoctyrium* sp., MarinBio, Turkey), Protein-Plus® (PP; 0.3–1 g/10^6 rotifers, Algamac® Aquafaune Bio-Marine Inc., Hawthorne, USA), Inactive Baker's Yeast® (INBY; 0.3–0.4 g/10^6 rotifers, Simbiyotek Inc., Turkey), spray-dried *Nannochloropsis oculata* (SDN; 0.5–0.6 g/10^6 rotifers), and freshly cultured *Nannochloropsis oculata* (FN; density of 10^7 cell/mL/10^6 rotifers). Each diet was tested in triplicate.

Rotifer growth determination and sampling procedures

Each experimental feed was tested in triplicate and applied at a daily ration of 0.8 g dry weight (DW) 10^6 rotifer/day. During the experiment, egg number, egg-carrying female number, and total rotifer biomass were daily calculated. Three 1-mL samples were collected from each rotifer culture, and Lugol solution was added in order to settle rotifers for counting total number of rotifers, female, and egg-carrying rotifers. Dry weight was determined in 5 mL of microalgal culture or 100 mL of rotifer culture samples filtered on previously weighed

precombusted Whatman GF/C fiberglass filters. Microalgae biomass was washed three times with 5 mL of 0.5 M ammonium formate in order to remove salts, whereas rotifers samples were rinsed with distilled water. Filters were dried overnight at 80 °C and dry weight determined gravimetrically. Microalgal biomass (25–50 mL) was sampled by centrifugation and immediately frozen at − 80 °C. For proximate, fatty acid and amino acid composition, 150 mL of rotifer culture was filtered on a sieve (45 μm mesh size), rinsed with distilled water, and frozen at − 80 °C until analysis.

Proximate and fatty acid analysis

Moisture (AOAC 2010), protein (AOAC 2010), and crude lipid (Folch et al. 1957) contents of rotifer diets were analyzed (Table 1).

Fatty acid methyl esters in microalgae, diets, and experimental rotifers were obtained by transmethylation with 1% sulfuric acid in methanol (Christie 1982). Fatty acid methyl esters were separated by GC (GC-14A; Shimadzu, Tokyo, Japan) in a Supercolvax-10-fused silica capillary column (constant pressure with 100 KPa, length 30 m; internal diameter 0.32 mm; 0.25 i.d (Ref.: 24080-U) Supelco, Bellefonte, PA, USA) using helium as a carrier gas. Column temperature was 180 °C for the first 10 min, increasing to 220 °C at a rate of 2 °C min^{-1}, and then held at 220 °C for 15 min. Fatty acid methyl esters were quantified by FID following the conditions described in Izquierdo et al. (1990) and identified by comparison with external standards and well characterized fish oils (EPA 28, Nippai, Ltd. Tokyo, Japan). Fatty acid profiles of rotifer diets are shown in Table 2.

Amino acid analysis

Total amino acids analyses were conducted at the Scientific and Technological Research Council of Turkey (Gebze, MAM, Turkey). From each sample, 5 mg was added to a glass ampoule together with 5-mL lithium citrate loading buffer. Ampoules were sealed and placed in an oven (115 °C) for 2 h to facilitate hydrolysis. The samples were then removed, allowed to cool, and filtered with a 0.45 PTFE syringe filter. Samples were diluted as necessary to fall within the detectable range for the assay. Amino acids were measured with a Biochrom 30 amino acid analyzer (Biochrom, Holliston, MA, USA) (Hawkyard et al. 2016) (Table 3).

Table 1 Proximate composition (g 100 g^{-1} dry weight) of experimental rotifer diets

Diets	Algome®[1]	PP®[2]	INBY®[3]	SDN[4]	FN[5]
Crude protein	16.75 ± 0.01	42.01 ± 0.03	45.24 ± 0.04	58.54 ± 0.03	42.04 ± 0.07
Crude lipids	0.41 ± 0.02	3.44 ± 0.04	1.01 ± 0.02	1.06 ± 0.05	5.91 ± 0.02
Crude ash	24.35 ± 0.59	6.93 ± 0.04	4.61 ± 0.01	10.27 ± 0.11	5.55 ± 0.09
Moisture	96.33 ± 0.04	93.84 ± 0.10	94.89 ± 0.37	97.24 ± 0.16	97.12 ± 0.07

[1] Algome (MarinBio Inc., Aydın, Turkey)

[2] Protein Plus (Aquafaune Bio-Marine Inc., Hawthorne, USA)

[3] Inactive Baker's Yeast (Simbiyotek Inc., Turkey)

[4] Spray-dried *Nannochloropsis oculata*

[5] Fresh *Nannochloropsis oculata*

Table 2 Main fatty acid compositions of experimental rotifer (*Brachionus plicatilis*) feeds (% total fatty acids)

	Algome®[1]	PP[2]	INBY[3]	SDN[4]	FN[5]
12:0	0.22 ± 0.00a	0.23 ± 0.01a	0.16 ± 0.00b	0.05 ± 0.00c	0.16 ± 0.00b
13:0	0.04 ± 0.00	n.d.	n.d.	n.d.	n.d.
14:0	6.07 ± 0.00d	8.94 ± 0.01c	0.60 ± 0.02e	1.05 ± 0.02b	1.43 ± 0.00a
14:1	n.d.	n.d.	0.11 ± 0.00	n.d	n.d.
15:0	2.60 ± 0.01c	0.34 ± 0.00b	0.26 ± 0.01c	0.50 ± 0.00a	0.49 ± 0.02a
16:0	52.02 ± 0.04a	25.61 ± 0.04c	18.80 ± 0.21d	28.45 ± 0.08b	25.94 ± 0.08c
16:1	n.d.	0.24 ± 0.01d	28.76 ± 0.18a	1.65 ± 0.02b	0.95 ± 0.03c
17:0	0.82 ± 0.00a	0.08 ± 0.01d	0.23 ± 0.02b	0.11 ± 0.01c	0.17 ± 0.00b
18:0	1.22 ± 0.01d	0.75 ± 0.01e	14.38 ± 0.05a	2.11 ± 0.02c	4.51 ± 0.00b
18:1n-9	1.55 ± 0.00d	0.95 ± 0.00e	28.21 ± 0.13a	4.83 ± 0.02c	15.71 ± 0.02b
18:2n-6	3.20 ± 0.01c	3.88 ± 0.01c	0.46 ± 0.01d	19.71 ± 0.03b	23.98 ± 0.05a
18:3n-3	0.08 ± 0.00d	0.36 ± 0.00c	n.d.	17.29 ± 0.06a	10.97 ± 0.03b
18:3n-6	0.03 ± 0.00b	1.12 ± 0.21a	n.d.	n.d.	n.d.
20:0	n.d.	0.09 ± 0.01c	0.14 ± 0.01b	0.14 ± 0.01b	0.95 ± 0.01a
20:1	n.d.	n.d.	0.09 ± 0.01c	0.18 ± 0.02b	0.42 ± 0.01a
20:2n-6	0.10 ± 0.01b	0.23 ± 0.00a	n.d.	0.10 ± 0.01b	0.09 ± 0.00b
20:1n-9	n.d.	n.d.	n.d.	0.98 ± 0.04	n.d.
20:3n-3	0.30 ± 0.00b	0.99 ± 0.01a	0.24 ± 0.00b	n.d	0.03 ± 0.01c
20:3n-6	0.06 ± 0.00b	0.29 ± 0.00a	n.d.	n.d.	n.d.
20:4n-6	0.04 ± 0.01c	0.28 ± 0.01a	n.d.	n.d.	0.10 ± 0.00b
20:5n-3	0.24 ± 0.01b	0.74 ± 0.00a	n.d.	n.d.	0.05 ± 0.01c
22:0	0.05 ± 0.01c	0.06 ± 0.01c	0.06 ± 0.01c	0.08 ± 0.00b	0.30 ± 0.01a
22:5n-6	n.d.	0.21 ± 0.01	n.d	0.09 ± 0.01	0.08 ± 0.01
22:6n-3	24.84 ± 0.01b	34.88 ± 0.01a	0.09 ± 0.02c	0.10 ± 0.08c	0.04 ± 0.00d
23:0	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.01
24:0	0.04 ± 0.00d	0.22 ± 0.01b	0.16 ± 0.01c	0.24 ± 0.00b	0.46 ± 0.00a
24:1	0.04 ± 0.00b	0.17 ± 0.01a	n.d.	n.d.	n.d.
Σ Saturated	63.07 ± 0.05a	36.29 ± 0.05b	34.79 ± 0.18c	32.71 ± 0.07c	34.63 ± 0.04c
Σ Monounsaturated	0.04 ± 0.00d	0.41 ± 0.02d	28.96 ± 0.16b	1.82 ± 0.04a	1.37 ± 0.04c
Σ n-3	25.45 ± 0.01b	36.96 ± 0.00a	0.33 ± 0.02e	17.39 ± 0.02c	11.08 ± 0.03d
Σ n-6	3.33 ± 0.02c	1.16 ± 0.05d	0.46 ± 0.01e	19.80 ± 0.04b	24.15 ± 0.06a
Σ n-9	1.55 ± 0.00d	0.95 ± 0.00d	28.21 ± 013a	5.81 ± 0.02c	15.71 ± 0.02b
Σ n-3 HUFA	25.37 ± 0.01b	36.60 ± 0.00a	0.33 ± 0.02c	0.10 ± 0.08d	0.11 ± 0.00d
EPA/ARA	6.83 ± 1.18a	2.65 ± 0.13b	n.d.	n.d.	0.45 ± 0.07c
DHA/EPA	105.73 ± 3.15a	47.13 ± 0.01b	n.d.	0.15 ± 0.00d	0.90 ± 0.14c
DHA/ARA	724.33 ± 146.14a	124.71 ± 6.27b	n.d.	0.15 ± 0.00d	0.40 ± 0.00c
n-3/n-6	7.65 ± 0.04b	32.00 ± 1.41a	0.71 ± 0.02d	0.88 ± 0.00c	0.46 ± 0.00e

Different letters show significant differences among groups

n.d. not detected

[1] Algome (MarinBio Inc., Aydın, Turkey)

[2] Protein Plus (Aquafaune Bio-Marine Inc., Hawthorne, USA)

[3] Inactive Baker's Yeast (Simbiyotek Inc., Turkey)

[4] Spray-dried *Nannochloropsis oculata*

[5] Fresh *Nannochloropsis oculata*

Statistical analysis

All data were statistically analyzed using a SPSS Statistical Software System 15.0. The significant level for all the analysis was set at 5%, and results were given as mean values and standard deviation. All values presented as percentage were arcsine-transformed. Also, all variables were checked for normality and homogeneity of variance, using the Kolmogorov–

Table 3 Free amino acid compositions (mg g^{-1} dry weight, mean ± SD) of experimental rotifer feeds

	Algome®[1]	PP®[2]	INBY®[3]	SDN[4]	FN[5]
Aspartic acid	5.48 ± 0.07b	13.27 ± 0.09a	2.77 ± 0.03c	1.98 ± 0.04c	0.46 ± 0.016d
Glutamic acid	8.65 ± 0.03b	12.01 ± 0.01a	4.53 ± 0.03c	4.49 ± 0.03c	0.83 ± 0.06d
Serine	2.18 ± 0.01a	1.83 ± 0.02b	1.51 ± 0.01c	1.74 ± 0.02b	0.25 ± 0.014d
Glysine	1.60 ± 0.02a	1.47 ± 0.04b	1.54 ± 0.010b	1.53 ± 0.01b	0.38 ± 0.02c
Histidine	1.31 ± 0.07a	0.79 ± 0.01b	0.71 ± 0.03b	0.80 ± 0.08b	0.06 ± 0.012c
Arjinine	2.34 ± 0.09a	1.29 ± 0.01c	0.83 ± 0.02d	1.66 ± 0.08b	0.37 ± 0.00e
Threonine	2.25 ± 0.04a	0.40 ± 0.00d	1.13 ± 0.08c	1.77 ± 0.09b	0.36 ± 0.04d
Alanine	3.19 ± 0.01a	2.47 ± 0.06b	2.06 ± 0.09c	1.98 ± 0.01c	0.52 ± 0.06d
Proline	1.53 ± 0.01bc	1.30 ± 0.03c	1.89 ± 0.02b	3.28 ± 0.09a	0.81 ± 0.05d
Tyrosine	1.67 ± 0.01a	1.48 ± 0.00b	1.56 ± 0.07b	1.04 ± 0.01c	0.26 ± 0.07d
Valine	3.05 ± 0.05a	1.88 ± 0.01c	2.72 ± 0.05b	1.77 ± 0.08c	0.41 ± 0.04d
Methionine	0.56 ± 0.02a	0.12 ± 0.01c	0.53 ± 0.01b	0.12 ± 0.00c	–
Isoleucine	2.36 ± 0.00a	1.35 ± 0.04c	2.00 ± 0.01b	1.12 ± 0.04c	0.25 ± 0.03d
Leucine	3.00 ± 0.02a	2.52 ± 0.06c	2.84 ± 0.03b	2.20 ± 0.01c	0.52 ± 0.06d
Phenylalanine	2.14 ± 0.02a	1.70 ± 0.03b	2.17 ± 0.07a	1.59 ± 0.09b	0.41 ± 0.02c
Lysine	3.75 ± 0.02a	3.83 ± 0.014a	3.38 ± 0.01b	3.55 ± 0.01b	0.51 ± 0.03c
Free amino acid	45.09 ± 0.05a	47.73 ± 0.03a	32.19 ± 0.02b	30.65 ± 0.07b	6.46 ± 0.04c
Essential amino acid	18.42 ± 0.07a	12.59 ± 0.05c	15.48 ± 0.04b	12.92 ± 0.03c	2.52 ± 0.08d
Non-essential amino acid	26.27 ± 0.02b	35.14 ± 0.01a	16.71 ± 0.04c	17.73 ± 0.04c	3.94 ± 0.02d

Different letters show significant differences among groups

[1] Algome (MarinBio Inc., Aydın, Turkey)

[2] Protein Plus (Aquafaune Bio-Marine Inc., Hawthorne, USA)

[3] Inactive Baker's Yeast (Simbiyotek Inc., Turkey)

[4] Spray-dried *Nannochloropsis oculata*

[5] Fresh *Nannochloropsis oculata*

Smirnov and the Levene's test respectively. Means were compared using one-way ANOVA or a Kruskal–Wallis test.

Results

Rotifer growth performance

Rotifers fed SDN showed significantly higher total biomass production during first 11 days of feeding ($P < 0.05$) (Fig. 2). Rotifers fed IBNY did not continue after 11 days of culture. Population of IBNY and Algome® groups started to decrease at 8–11 days of culture, whereas PP and FN groups similarly increased but were still significantly lower than that of SDN group. At 12 and 13 days of culture, there were no significant differences among the rotifers fed PP, SDN, FN, and those three groups showed higher biomass production than rotifers fed Algome® diet. FN showed significantly increasing potential of total rotifer biomass in the last 3 days of feeding trial ($P < 0.05$) (Fig. 2).

Rotifer fed SDN showed higher egg production (per mL) during first 11 days of experiment ($P < 0.05$) (Fig. 3). FN group rotifers significantly contained higher number of eggs at 12 (350 eggs/mL), 15 (180 eggs/mL), and 16 (170 eggs/mL) days of experiment ($P < 0.05$) (Fig. 3).

Rotifers fed SDN showed significantly higher egg-carrying female number from day 1 to day 12 of culture ($P < 0.05$) (Fig. 4). Rotifers fed FN showed highest value of egg-carrying

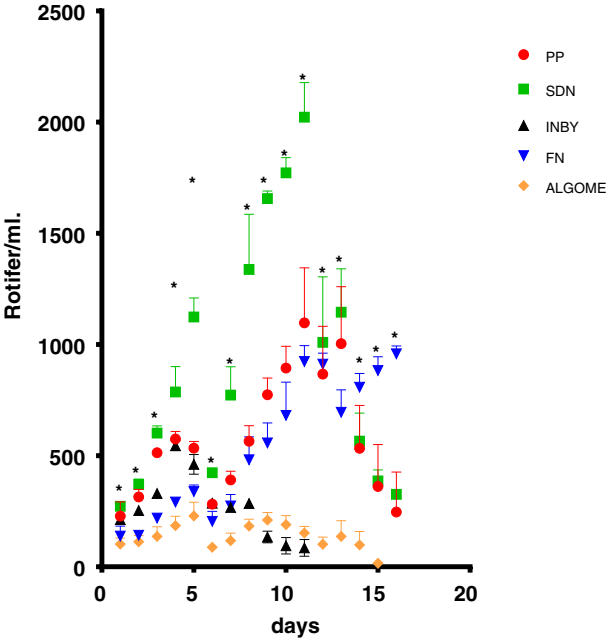

Fig. 2 Density rotifer fed different feeds during 16 days (asterisk means significant differences among treatment, $P < 0.05$)

female number at 12 days of culture. At day 13 of the culture period, only rotifer group fed Algome® showed the lowest value. Rotifers fed SDN and FN showed significantly higher

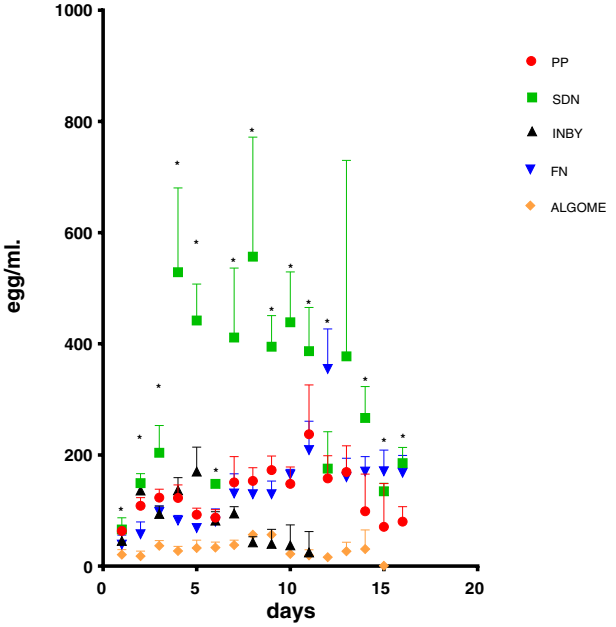

Fig. 3 Rotifer egg density (egg/mL, mean ± SD) fed different feeds during 16 days experiment

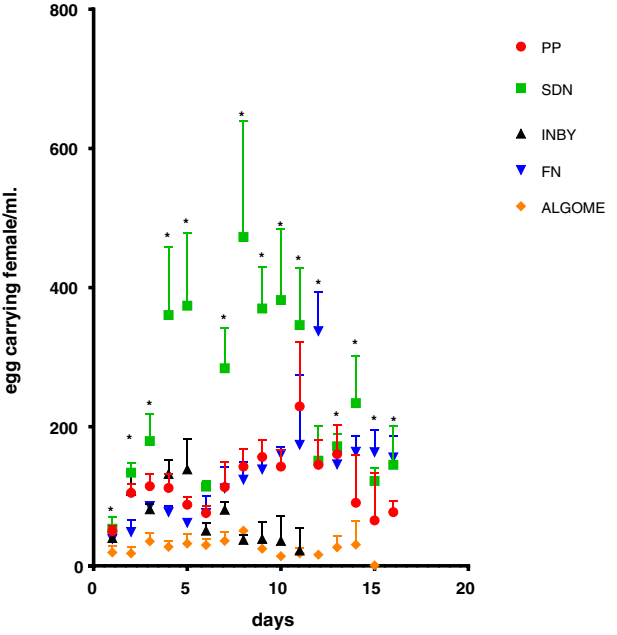

Fig. 4 Density of egg-carrying female (egg-carrying female/mL, mean ± SD) fed different feeds during 16 days

egg-carrying female numbers for the last 3 days of culture ($P < 0.05$). Female rotifers were only observed after 11 days of culture in INBY group.

Proximate and fatty acid composition of rotifers

At the end of the feeding trial, rotifer fed Protein Plus diet presented significantly ($P < 0.05$) higher crude protein content than the other groups (Table 4).

Similarly, rotifer fed Protein Plus® diet showed significantly ($P < 0.05$) higher crude lipid content than the other groups. At the end of the feeding, arachidonic acid (ARA; 20:4n-6),

Table 4 Proximate compositions (g 100 g^{-1} dry weight) of rotifer *Brachionus plicatilis* fed different diets at the end of the experiment. Values expressed in mean ± SD ($n = 3$ tanks/diet)

	Initial[1]	Algome®[2]	PP®[3]	INBY®[4]	SDN[5]	FN[6]
Crude protein	37.07 ± 0.01	42.25 ± 0.02b	45.04 ± 0.07a	40.03 ± 0.06c	40.01 ± 0.02c	38.02 ± 0.08d
Crude lipid	0.44 ± 0.08	0.60 ± 0.13d	1.43 ± 0.18a	0.89 ± 0.12c	1.29 ± 0.48b	1.41 ± 0.05a
Crude ash	1.15 ± 0.01	1.67 ± 0.03b	1.21 ± 0.02d	1.23 ± 0.01d	1.33 ± 0.03c	1.94 ± 0.05a
Dry matter	14.48 ± 1.04	10.20 ± 0.14d	12.23 ± 0.02b	12.98 ± 0.15a	11.64 ± 1.17c	8.67 ± 0.11e

Different letters within a line denote significant differences ($P < 0.05$)

[1] Initial (rotifer biomass at the beginning of experiment)

[2] Algome (MarinBio Inc., Aydın, Turkey)

[3] Protein Plus (Aquafaune Bio-Marine Inc., Hawthorne, USA)

[4] Inactive Baker's Yeast (Simbiyotek Inc., Turkey)

[5] Spray-dried *Nannochloropsis oculata*

[6] Fresh *Nannochloropsis oculata*

eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), ∑n-3, and ∑n-3 HUFA increased in rotifers by feeding Protein Plus® ($P < 0.05$). Similarly, DHA/EPA ratio and n-3/n-6 ratio were higher in rotifers fed Protein Plus® ($P < 0.05$). However, oleic acid level was found higher in rotifer fed Inactive Baker's Yeast ($P < 0.05$). Moreover, linoleic acid (LA; 18:2n-6) was found higher in rotifers fed spray-dried *Nannochloropsis oculata*. α-Linolenic acid (LNA; 18:3n-3) was enhanced by both diets, spray-dried *Nannochloropsis oculata* and fresh *Nannochloropsis oculata* ($P < 0.05$) (Table 5).

Amino acid composition of rotifers

At end of the feeding trial, essential amino acid composition of rotifers showed that histidine (Hist.), threonine (Thr.), tyrosine (Tyr.), valine (Val.), methionine (Meth.), isoleucine (Iso.), leucine (Leu.), phenylalanine (Phe.), and lysine (Lys.) were significantly higher in rotifers fed INBY ($P < 0.05$). On the contrary, aspartic acid, glutamic acid, serine, glycine, alanine, and proline were higher in rotifers fed spray-dried *Nannochloropsis oculata* ($P < 0.05$). Arginine contents in rotifer were increased by feeding fresh *Nannochloropsis oculata* ($P < 0.05$) (Table 6).

Discussion

The results of this study showed that spray-dried form of the microalgae *Nannochloropsis oculata* is an optimal feed for rotifer production at commercial scale but requires a final enricher following high biomass production. *N. oculata* is commonly used for greenwater (Skiftesvik et al. 2003; Rocha et al. 2008), rotifer production (Ferreira et al. 2009), or as feed ingredient in early weaning diets for marine fish larviculture (Van der Meeren et al. 2007; Eryalçın et al. 2013; Cavonius et al. 2015; Eryalçın et al. 2015). Due to their high nutritional value, suitable size, and high productivity potential, this type of small size microalgae is still highly required for rotifer and marine fish larvae culture (Reitan et al. 1997; Shields and Lupatsch 2012). Marine microalgae have a high content of lipid, protein, and pigment (Borowitzka 2013). They are processed in several ways and used as enrichment for rotifer (Ferreira et al. 2009), *Artemia* (Ma and Qin 2014), and copepods (Qie et al. 2011; Knuckey et al. 2005; Rasdi and Qin 2016). They are also used as alternative protein and lipids ingredients in larval microdiets (Ganuza et al. 2008; Walker and Berlinsky 2011; Ju et al. 2012; Patterson and Gatlin 2013; Eryalçın et al. 2013; Eryalçın et al. 2015). However, the difficulty to optimize the nutritional value of fresh microalgae is still an issue to overcome depending on culture medium, harvesting time, labor talent, and the physical and chemical conditions of culture water (Cho et al. 2007). Therefore, several dried commercial rotifer feeds are widely used due to their high nutritional value and long-lasting duration in high biomass culture (Vigani et al. 2015). Nevertheless, these products are expensive and commercial hatcheries still look for alternative feeding materials. This study suggests the combined usage of an alternative dried form, together with an enricher for optimal quality and quantity.

Rotifer culture management should sustain satisfactory amounts for successful marine fish larvae production in commercial hatcheries. In this study, rotifers fed spray-dried *Nannochloropsis oculata* diet resulted in significantly higher rotifer biomass during 16 days of semi-continuous culture, with an increasing biomass trend that lasted 11 days, high egg production, and egg-carrying female numbers, showing that SDN can be adequately used to replace other commercial feeds.

Table 5 Fatty acid composition of rotifers fed different diets (% dry weight, mean ± SD, $n = 3$)

	Initial[1]	Algome®[2]	PP®[3]	INBY®[4]	SDN[5]	FN[6]
10:0	0.09 ± 0.01	n.d.	n.d.	0.11 ± 0.01	n.d.	0.04 ± 0.00
12:0	1.37 ± 0.03	0.21 ± 0.01b	0.22 ± 0.00b	0.60 ± 0.01a	0.10 ± 0.00c	n.d.
13:0	n.d.	n.d.	n.d.	n.d.	n.d.	0.04 ± 0.01
14:0	1.95 ± 0.02	4.48 ± 0.04b	5.38 ± 0.01a	3.02 ± 0.06c	2.49 ± 0.07e	3.22 ± 0.01d
14:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15:0	0.37 ± 0.02	1.89 ± 0.02a	0.62 ± 0.00e	0.90 ± 0.01d	1.21 ± 0.04b	1.14 ± 0.03c
16:0	10.99 ± 0.04	22.74 ± 0.12b	16.16 ± 0.00d	11.08 ± 0.02e	23.10 ± 0.06a	21.30 ± 0.01c
16:1	16.68 ± 0.06	6.26 ± 0.04b	2.37 ± 0.02c	16.61 ± 0.06a	2.07 ± 0.02d	1.87 ± 0.01e
17:0	0.23 ± 0.01	0.62 ± 0.03a	0.28 ± 0.01c	0.58 ± 0.00b	0.29 ± 0.01c	0.33 ± 0.01d
18:0	5.04 ± 0.07	4.17 ± 0.01b	2.49 ± 0.01e	5.88 ± 0.02a	2.92 ± 0.02c	2.73 ± 0.01d
18:1n-9	32.03 ± 0.13	8.38 ± 0.00b	4.26 ± 0.10e	23.76 ± 0.08a	6.41 ± 0.01c	4.82 ± 0.01d
18:2n-6	3.45 ± 0.08	6.20 ± 0.07d	11.67 ± 0.05c	2.76 ± 0.10e	22.56 ± 0.16a	21.76 ± 0.01b
18:3n-3	0.51 ± 0.02	0.26 ± 0.07c	1.21 ± 0.01b	n.d.	12.49 ± 0.03a	12.40 ± 0.03a
18:3n-6	n.d.	n.d.	1.13 ± 0.03	n.d.	n.d.	n.d.
20:0	0.14 ± 0.01	0.20 ± 0.00b	0.13 ± 0.00d	0.24 ± 0.00a	0.12 ± 0.01d	0.17 ± 0.02c
20:1	2.66 ± 0.03	0.72 ± 0.06d	0.63 ± 0.01e	2.25 ± 0.02a	1.11 ± 0.02c	1.43 ± 0.01b
20:2n-6	n.d.	0.30 ± 0.01c	1.35 ± 0.07d	n.d.	1.93 ± 0.01a	1.72 ± 0.06b
20:1n-9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20:3n-3	n.d.	0.15 ± 0.04c	0.88 ± 0.00b	0.10 ± 0.00d	1.13 ± 0.01a	n.d.
20:3n-6	0.31 ± 0.02	0.33 ± 0.00c	1.51 ± 0.00a	n.d.	0.89 ± 0.01b	0.91 ± 0.01b
20:4n-6	0.70 ± 0.04	0.78 ± 0.30b	1.75 ± 0.01a	n.d.	n.d.	0.34 ± 0.04c
20:5n-3	0.32 ± 0.00	2.02 ± 0.01b	2.54 ± 0.01a	0.21 ± 0.05d	0.15 ± 0.01d	0.28 ± 0.01c
22:0	0.13 ± 0.01	0.23 ± 0.00b	0.15 ± 0.01c	0.26 ± 0.01a	0.12 ± 0.01d	0.11 ± 0.00e
22:5n-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d..
22:1n-9	0.92 ± 0.01	0.23 ± 0.01	0.28 ± 0.00	0.70 ± 0.01	0.32 ± 0.04	n.d.
22:5n-6	n.d.	1.13 ± 0.08a	1 ± 0.00b	n.d.	n.d.	n.d.
22:6n-3	n.d.	9.78 ± 0.27b	21.74 ± 0.19a	n.d.	n.d.	n.d.
23:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24:0	n.d.	0.28 ± 0.01a	0.18 ± 0.01d	0.24 ± 0.00b	0.21 ± 0.01c	0.14 ± 0.00e
24:1	0.59 ± 0.04	n.d.	n.d.	0.67 ± 0.11a	0.42 ± 0.35b	0.31 ± 0.03c
Σ Saturated	20.29 ± 0.00	34.81 ± 0.17a	25.60 ± 0.02d	22.98 ± 0.04e	30.54 ± 0.16b	29.20 ± 0.01c
Σ Monounsaturated	19.93 ± 0.01	6.97 ± 0.03b	2.99 ± 0.01d	19.53 ± 0.04a	3.59 ± 0.40c	3.61 ± 0.05c
Σ n-3	0.83 ± 0.02	12.20 ± 0.17d	26.36 ± 0.21a	0.31 ± 0.05e	13.77 ± 0.01b	12.68 ± 0.04c
Σ n-6	4.45 ± 0.10	8.43 ± 0.16d	16.05 ± 0.03c	2.76 ± 0.10e	23.45 ± 0.18a	23.00 ± 0.02b
Σ n-9	32.95 ± 0.15	8.61 ± 0.01b	4.54 ± 0.10e	24.46 ± 0.08a	6.73 ± 0.02c	4.82 ± 0.01d
Σ n-3 HUFA	0.32 ± 0.00	11.94 ± 0.24b	25.16 ± 0.21a	0.31 ± 0.05d	1.28 ± 0.02c	0.28 ± 0.01d
EPA/ARA	n.d.	2.81 ± 1.10a	1.46 ± 0.00b	0.31 ± 0.05b	0.15 ± 0.01b	0.84 ± 0.05b
DHA/EPA	n.d.	4.85 ± 0.12b	8.56 ± 0.03a	n.d.	n.d.	n.d.
DHA/ARA	n.d.	13.60 ± 4.99	12.46 ± 0.06	n.d.	n.d.	n.d.
n-3/n-6	0.19 ± 0.00	1.45 ± 0.01b	1.64 ± 0.01a	0.11 ± 0.02e	0.59 ± 0.00c	0.55 ± 0.00d

Different letters show significant differences among rotifers fed different diets in selected fatty acids

n.d. not detected

[1] Initial (rotifer biomass at the beginning of experiment)

[2] Algome (MarinBio Inc., Aydın, Turkey)

[3] Protein Plus (Aquafaune Bio-Marine Inc., Hawthorne, USA)

[4] Inactive Baker's Yeast (Simbiyotek Inc., Turkey)

[5] Spray-dried *Nannochloropsis oculata*

[6] Fresh *Nannochloropsis oculata*

Marine fish larvae require sufficient amount of essential fatty acids for their growth and survival (Izquierdo and Koven 2011). Those essential compounds should be delivered via live feeds to larvae. Commonly used microalgae *Nannochloropsis oculata* is known to be rich in

Table 6 Free amino acid compositions (mg g^{-1} dry weight, mean ± SD, $n = 3$, different letters denote significant differences among groups) of rotifer biomass fed different feeds

	Initial[1]	Algome®[2]	PP®[3]	INBY®[4]	SDN[5]	FN[6]
Aspartic acid	0.57 ± 0.11	0.07 ± 0.15d	1.15 ± 0.03b	1.10 ± 0.04c	1.32 ± 0.02a	1.14 ± 0.09bc
Glutamic acid	0.67 ± 0.01	1.33 ± 0.16d	1.66 ± 0.14c	1.71 ± 0.07b	1.85 ± 0.07a	1.65 ± 0.01c
Serine	0.23 ± 0.01	2.36 ± 0.01d	0.24 ± 0.02c	0.32 ± 0.02a	0.32 ± 0.02a	0.25 ± 0.00b
Glycine	0.23 ± 0.07	0.23 ± 0.14d	0.27 ± 0.00c	0.29 ± 0.01b	0.29 ± 0.02a	0.26 ± 0.00c
Histidine	0.10 ± 0.03	0.11 ± 1.41c	0.10 ± 0.02c	0.16 ± 0.01a	0.14 ± 0.05b	0.11 ± 0.02c
Arginine	0.37 ± 0.14	0.38 ± 0.05d	0.47 ± 0.0b	0.47 ± 0.04b	0.45 ± 0.03c	0.62 ± 0.02a
Threonine	0.23 ± 0.00	0.12 ± 0.00e	0.15 ± 0.02c	0.21 ± 0.00a	0.20 ± 0.02b	0.13 ± 0.02d
Alanine	0.33 ± 0.00	0.23 ± 0.28d	0.29 ± 0.05c	0.33 ± 0.03b	0.36 ± 0.05a	0.29 ± 0.01c
Proline	0.28 ± 0.07	0.22 ± 0.01c	0.25 ± 0.01b	0.30 ± 0.012a	0.31 ± 0.01a	0.28 ± 0.01b
Tyrosine	0.26 ± 0.07	0.12 ± 0.03e	0.27 ± 0.07d	0.32 ± 0.04a	0.28 ± 0.02c	0.28 ± 0.00b
Valine	0.32 ± 0.02	0.24 ± 0.4d	0.33 ± 0.02c	0.4 ± 0.015a	0.36 ± 0.03b	0.31 ± 0.02c
Methionine	0.07 ± 0.00	0.004 ± 0.00d	–	0.05 ± 0.01a	0.02 ± 0.00c	0.03 ± 0.00b
Isoleucine	0.30 ± 0.03	0.21 ± 0.02d	0.30 ± 0.02c	0.38 ± 0.04a	0.32 ± 0.03b	0.29 ± 0.02c
Leucine	0.45 ± 0.07	0.31 ± 0.02e	0.44 ± 0.00d	0.53 ± 0.00a	0.52 ± 0.00b	0.46 ± 0.00c
Phenylalanine	0.32 ± 0.07	0.20 ± 0.2e	0.32 ± 0.00d	0.38 ± 0.04a	0.36 ± 0.02b	0.33 ± 0.01c
Lysine	0.81 ± 0.01	0.49 ± 0.07d	0.82 ± 0.01c	1.18 ± 0.03a	1.06 ± 0.08ab	1.03 ± 0.00b
Free amino acid	5.61 ± 0.01d	5.18 ± 0.02d	7.06 ± 0.04c	8.15 ± 0.01a	8.17 ± 0.05a	7.49 ± 0.07b
Essential amino acid	2.6 ± 0.06c	1.72 ± 0.04d	2.46 ± 0.08c	3.61 ± 0.04a	2.98 ± 0.01b	2.69 ± 0.09c
Non-essential amino acid	3.01 ± 0.01e	3.46 ± 0.02d	4.6 ± 0.02bc	4.54 ± 0.05c	5.19 ± 0.09a	4.8 ± 0.01b

[1] Initial (rotifer biomass at the beginning of experiment)

[2] Algome (MarinBio Inc., Aydın, Turkey)

[3] Protein Plus (Aquafaune Bio-Marine Inc., Hawthorne, USA)

[4] Inactive Baker's Yeast (Simbiyotek Inc., Turkey)

[5] Spray-dried *Nannochloropsis oculata*

[6] Fresh *Nannochloropsis oculata*

EPA but lacking DHA. In this study, rotifer fed both *Nannochloropsis* feeds (SDN and FN) did not contain sufficient amount of essential fatty acids for marine fish larvae, compared to those fed the two other diets. According to our results, essential fatty acids (ARA, EPA, and DHA) and total n-3 levels were supported best by utilization of PP®. Proper ratio of n-3 to n-6 fatty acids is also important for optimal growth in marine fish larvae. In this study, the highest n-3/n-6 ratio (1.64) was obtained in rotifer fed PP® diet, with values similar to those in enriched rotifer (Bransden et al. 2005). According to fatty acid composition, cultivation with PP diet does not require fatty acid enrichments for rotifers. On the other hand, microalgae diets seem to require enrichment during cultivation. One of the reasons that microalgae based feeds did not support well the fatty acid profiles could be the different fatty acid conversion metabolisms in rotifers, compared to copepods. Copepods apparently have the ability to convert 18:3n-3 into 20:5n-3 and 22:6n-3 (Nanton and Castell 1999) while rotifers cannot (Seychelles et al. 2009; Haché and Plante 2011); this event is related to desaturating capacity of selected copepod species, cultured temperature, and given feeds.

Free amino acids, histidine, arginine, glycine, and alanine play important roles in survival, growth, and physiological functions such as protection against pH change, neurological function and development, cell signaling, blood flow, appetite of larval, and juvenile marine fish (Conceição et al. 2003; Li et al. 2009). Several studies show that commercial products support well the AA levels in rotifers; for example, Rocha et al. (2017) reported that enriched rotifers with

Ori-green® and protein hydrolysate resulted in the accumulation of higher DHA, EPA/ARA, free amino acid, and valine levels in rotifer body in short time. Similarly, AA levels in INBY and PP diets in this study were well reflected to rotifers. According to EAA composition of rotifer diets, Algome® seems to be the best AA source (Srivastava et al. 2006). In this study, despite the highest AA composition among diets was in Algome®, it was not reflected to rotifers. On the other hand, AA composition in INBY and SDN diets which were lower than that in Algome® was effectively reflected to rotifers, giving rise to the highest protein content and highest growth among experimental groups. When amino acid content of successful diet SDN is considered, proline content was significantly higher compared to other groups. Proline play an important role in the antioxidant activity of peptides; this fact could have supported the utilization of amino acids which might have resulted in higher growth (Chen et al. 1996).

From the point of view of commercial marine fish hatcheries, growth rate and nutritional value of rotifer should be enhanced in order to sustain both quantity and quality of cultured marine fish species (Ma and Qin 2014; Maisashvili et al. 2015). For that reason, several artificial diets for rotifers are used commonly in order to ease utilization and formulation. Artificial macroalgal detritus, for example, results in lower growth when utilized alone but attains the same growth level with fresh *Nannochloropsis* sp. when utilized in combination with fresh microalgae in equal amounts (Yin et al. 2013). Similarly, flocculated and paste form of *Nannochloropsis oculata* performed better than commercial products for juvenile seahorse culture (Sales et al. 2016). Park et al. (2006) evaluated different types of spray-dried microalgae for enrichment of rotifers during 24 h. According to that study, dried *Crypthecodinium cohnii*– and *Schizochytrium* sp.–enriched rotifers resulted in better growth in cod larvae due to the high level of DHA. SDN in this study sustained effectively rotifer growth, however, when nutritional properties are considered, feeding rotifer with SDN do not seem ideal. Our study demonstrates that SDN is an optimal feed as long as rotifers are enriched with an EFA source after cultivation. According to our results, it is recommended to use a good rotifer food, followed by an enrichment product that optimizes the use of n-3 HUFA. Similarly, the combined utilization of fresh microalgae *Pavlova* sp. paste and commercial product Algamac 2000® seem to give an appropriate fatty acid profile for marine fish larvae (Garcia et al. 2008).

In our study, one of the reasons that fresh *Nannochloropsis oculata* did not perform as well as in SDN could be related to higher ash content in fresh form and different physicochemical properties that make the nutrients less available than those of spray-dried microalgae. Rotifer is a filter feeding organism and the ingestion rate of feeds is directly related to the size of feeds (Dhont et al. 2013). Ingestion of nutritional compounds plays an important role for rotifer growth in batch culture (Rothhaupt 1995; Cheng et al. 2011). Therefore, selection of food type and size in rotifer feeding is also important in order to ensure ingestion of feeds by rotifers. The spray-drying process reduces microalgae sizes (Ryckebosch et al. 2011) which might potentially ease ingestion. Therefore, processed microalgae meal seems to be more convenient for widespread utilization in hatcheries.

Conclusion

Hatchery produced spray-dried *Nannochloropsis oculata* is the best option as an alternative rotifer feed for providing longer rotifer cultivation performance and high biomass. Additionally, once the desired production number is obtained, nutritional properties of rotifers should be enhanced by Protein Plus. Further study should focus on the combined utilization of both rotifer diets as final enrichment, Protein Plus (EFA source) and Inactive Baker's Yeast (EAA

source), potentially providing together a balanced and supported feed in terms of protein and lipid content.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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