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RESEARCH PAPER

Effects of Different Commercial Feeds and Enrichments on Biochemical Composition and Fatty Acid Profile of Rotifer (*Brachionus Plicatilis*, Müller 1786) and *Artemia Franciscana*

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Abstract

The objective of the present study was to determine the effects of several commercial rotifer feeds and enrichments on growth, biochemical and fatty acid composition of L-type rotifer and *Artemia franciscana* nauplii. In experiment I, five experimental diets (M0 Plus, S.Parkle, w-3 Yeast 60, Beaker's yeast and *Chlorella vulgaris*) were tested for rotifer culture performance and in the second experiment six enrichers (w-3 Olio, n-3 Top Rich, Red Pepper, Culture Selco, microalgae mixture (*Dunaliella salina* + *Chlorella vulgaris*) and Emulsion T) were evaluated for the fatty acid composition of rotifer and *Artemia franciscana*. In experiment I, rotifers fed S.Parkle and Beaker's yeast showed better biomass production and egg density while the number of egg carrying female number was higher. In experiment II, rotifers enriched n-3 Top Rich showed better n-3 HUFA retention whereas *Artemia franciscana* nauplii enriched Red Pepper showed highest HUFA accumulation. Culture Selco seems optimal for artemia enrichment for EPA (20:5n-3) and DHA (22:6n-3) accumulation. In conclusion, Beaker's yeast is still applicable in comparison to other commercial feeds in rotifer culture. Further study is needed for determination of mineral composition of rotifer feeds and commercial enrichments and their retention in live prey and larvae use in Turkey.

Keywords: Rotifer, artemia, growth, enrichment, fatty acids.

Introduction

Successful production of marine fish larvae is limited by the poor achivement of hatching rate and lack of essential nutrients in live prey and microdiets (Izquierdo & Koven, 2011). Live prey are necessary in order to stimulate digestive enzymes and sustain enough energy from exogenous feeds (Kanazawa, 2003; Hamre, Yufera, Rønnestad, Boglione & Conceiçao & Izquierdo, 2013). Therefore, utilization of rotifers and *Artemia* has high importance at larval stage, since feeding with dry diets delays gut development at that stage (Sargent, McEvoy, Estevez, Bell, Bell, Henderson & Tocher, 1999; Sorgeloos, Dhert, & Candreva, 2001; Hamre, Srivastava, Rønnestad, Mangor-Jensen & Stoss, 2008). However, rotifer and artemia are known to lack essential nutrients such as polyunsaturated fatty acids (PUFA), essential amino acids (EAA), vitamins and minerals (Hamre, Srivastava, Rønnestad, Mangor-Jensen & Stoss, 2008; Hamre, 2011). For that reason, live prey should be enriched with essential nutrients before they are given to fish larvae (Ferreira, Maseda, Fábregas, & Otero, 2008; Castillo, Gapasin, & Leaño, 2009; Demir & Diken, 2011; Mahre, Hamre, & Elvevoll, 2012). Nutritional composition of live prey is differed among hatcheries and this is an actual topic of recent researches (Hamre, 2016).

The highly unsaturated fatty acids (HUFA) such as docosahexaenoic (DHA;22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (ARA; 20:4n-6) have important functions in marine fish and crustacean metabolism (Izquierdo, Socorro, Arantzamendi, & Hernández-Cruz, 2000; Izquierdo, 2005). Another essential nutrient, minerals are becoming more important in enrichment and feeding process of live prey. Most popular rotifer feed, beaker's yeast has negative effect on nutritional value of rotifers (Lie, Haaland, Hemre, Maage, Lied, Rosenlund, Sandnes, & Olsen, 1997; Hamre, 2016) whereas freshly cultured microalgae are a common enricher to obtain good nutritional profile in hatcheries. Due to difficulties in manipulation and culture of microalgae, commercial products are widely used for feed and enrichment process. For instance, enrichment with *Spirulina* increased amino acid levels in artemia (Bhavan, Devi, Shanti, Radhakrishnan, & Poongodi, 2010) by which

important amino acids such as taurine could be transferred to larvae. Taurine is known as a free organic acid that plays important role in lipid digestion, absorption and osmoregulation (Kim, Matsunari, Takeuchi, Yokoyama, Murata, & Ishihara, 2007; Takeuchi, 2014). The taurine concentration of rotifer and artemia are lower than that of copepods (Van der Meeren, Olsen, Hamre, & Fyhn, 2008). Therefore, one of the experimental group was designed as Taurine source.

Several rotifer diets and enrichments are available on the market with different nutritional compositions. The products tested in our experimental groups are commonly used for enrichment process of live prey in Turkey. However, the effects of those products on fatty acid composition were not yet reported in details. The aim of the present study was to determine the effects of commonly used rotifer diets on rotifer culture performance (Experiment I); M0 Plus®, S.parkle®, w-3 yeast 60®, Beaker's yeast® and *Chlorella vulgaris* and the effects of several enrichments; Olio w-3®, Red Pepper®, Top Rich®, Culture Selco®, mixture of freshly cultured microalgae species (*Dunaliella salina* and *Chlorella vulgaris)* and Taurine emulsion (Emulsion T) (Experiment II) were evaluated on growth, biochemical and fatty acid compositions of rotifer and artemia.

Materials and Methods

Microalgae Culture

The original strain of *Dunaliella salina* and *Chlorella vulgaris* were provided by the Culture Collection of Algae and Protozoa (CCAP) culture collection. Microalgae culture were started in test tubes (30 ml.) kept under 22°C at 40 µE $m^{-2}s^{-1}$ light intensity, 12/12 light-dark photoperiod for 25 days. Culture volume was increased to test tubes to 5 lt. Erlenmeyer's flasks at density of 2.10^6 cell ml^{-1}. Culture flasks containing 2 lt. sterilized f/2 medium inoculated with 2 test tube stock cultures of *Dunaliella salina* at begining. Growth of microalgae culture was determined by using Neubaeur counting chamber each day. All cultures were carried out in triplicates and means are presented. 3N-BBM+V medium was used for *Chlorella vulgaris* culture described by CCAP (Scotland, UK).

Preparation of Emulsion T

Type-A gelatin (from porcine skin, 75-100 bloom; Sigma, St. Louis, MO) was dissolved in water at 50°C to produce a 1% (wt/wt) solution (Ando, Abe, Ookubo, & Namikawa, 2005). After the solution was cooled to room temperature, fish oil (Capelin oil, Denofa, Fredrikstad, Norway; 3 g), Taurine (Taurine Acros, New Jersey, USA; 1 g), vitamin B_1 and B_{12} (Sigma Aldrichi, Steinheim, Germany; 0.01 g) were homogenized with 40 ml. of this solution for 3 minutes using a four-blade blender at 15.000 rpm. Resulting emulsions were immediately used for the following enrichment.

Rotifer Stock Culture

Rotifers (*Brachionus plicatilis,* L-strain, mean lorica length 195.5 µm) were obtained from Kılıç Sea Products Inc., Güvercinlik Hatchery, Bodrum, Muğla, Turkey. Rotifers were cultured in two 135-L conical tanks at room temperature under continuous illumination during 24 hours at laboratory. Stock rotifers were fed daily at 9:00 a.m. with *Nannochloropsis gaditana* at 8×10^6 cells/ml. concentration and 04:00 p.m. at a level of 0.03 g L^{-1} instant Baker's yeast (*Saccharomyces cerevisiae*). Rotifers were cultivated in semi-continuous cultures. The water temperature (21.2 ± 0.5°C) and salinity (‰ 25) were measured daily during cultivation of stock culture. Rotifers were starved for one 12 hours and washed with sterilized seawater before experiments started.

Experiment I: Rotifer Feeding Experiment

At the beginning of the rotifer feeding experiment, when rotifer concentration reached 350 rotifer/ml in circular flat bottom 135 liters fiberglass stock tank, they were distributed into fifteen experimental conical glass flasks (10 lt.) reprensent five experimental groups in triplicate. Five experimental rotifer feeds were evaluated for 8 days feeding. The treatments for first experiment were 1) M0 Plus® (0.40 g for 10^6 ind. ml^{-1}), 2) S.parkle® (0.65 g for 10^6 ind. ml^{-1}), 3) w-3 yeast 60® (0.50 g for 10^6 ind. ml^{-1}), 4) Beaker's yeast® (0.5 g for 10^6 ind. ml^{-1}) and 5) *Chlorella vulgaris*. Each experimental feed was tested in triplicate and applied at a daily ration of 0.8 g dry weight (DW) 10^6 rotifer. day^{-1}. During the experiment, egg number, juvenile number, egg carrying female number and total rotifer biomass were counted and calculated every day. The sampling consisted of taking 3 times one milliliter sample collected with a glass pipette from each treatment and add Lugol solution in order to settle rotifers for counting.

Experiment II: Rotifer and Artemia Enrichment

Rotifer Enrichment

Thirty million rotifers were harvested from the stock culture tank (stock density was 400 rotifer ml^{-1}) for enrichment experiment. Initial rotifer density was 60 rotifer ml^{-1} in each treatment parallels. Rotifers were enriched with five enrichment products twice a day at 7 a.m. and 7 p.m. based on recommendation by products manufacturer and two of them were prepared in laboratory as microalgae mixture and Emulsion T

(lipid and Taurine mixture). Amount of enrichment products were calculated according to rotifers density in each experimental 1000 ml flask and all enrichers were homogenized using hand blender for 2 minutes. Calculation for each enrichers was as follows; a) 600 mg L^{-1} of Olio w-3® (Bernaqua, Belgium) b) 180 mg L^{-1} of Red Pepper® paste (Bernaqua, Belgium) c) 150 mg L^{-1} of (n-3) Top Rich® (Rich S.A., Greece) d) a mix of 2×10^6 cells/ml. *Chlorella vulgaris* and cells/ml. *Dunaliella salina* e) 0.8 g L^{-1} of Emulsion T. Rotifer biomass (25 gram) were harvested on 75 µm plankton nets after 24 hours, rinsed with sterilized seawater for 3 minutes, then stored in -80°C for biochemical and fatty acid analysis. Each treatment was carried out in triplicate.

Artemia Enrichment

Artemia franciscana metanauplii were obtained from the hatching of Great Salt Lake *Artemia* cysts (INVE Aquaculture Nutrition, Dendermonde, Belgium). Cysts were decapsulated as described by Bruggeman *et al.* (1980), then incubated during 24 h in 5 L cylindrical-conical glass tank containing seawater (38 g L^{-1} salinity) at 28°C. Gentle aeration was placed in bottom of the glass tank with continuous illumination. After hatching, Instar I nauplii were moved into cylindrical containers at 23 °C and rinsed with UV-treated seawater for 20 minutes. Artemia enrichment was conducted in 10 L conical glass container at a density of 300.000 individuals L^{-1} density at 22°C with moderate aeration. Six enrichment procedures were evaluated and artemia nauplii groups were enriched twice a day at 7 a.m. and 7 p.m. with following procedures; a) 250 mg L^{-1} of Olio w-3® (Bernaqua, Belgium) b) 750 mg L^{-1} Red Pepper® paste (Bernaqua, Belgium) c) 300 mg L^{-1} of (n-3) Top Rich® (Rich S.A., Greece) d) 0.1 g L^{-1} Culture Selco® (INVE Aquaculture, Belgium) e) a mix of *Chlorella vulgaris* and *Dunaliella salina* concentration of 3:1, respectively (from stock concentration 5.75×10^6, 1.25×10^6 cell/ml.) and f) 0.8 g L^{-1} Emulsion T. All enriched artemia were harvested (40 g from per treatment) after 24 h on 125 µm filter and rinsed with autoclaved seawater for 5 min then stored in -80°C for biochemical and fatty acid analysis. Each treatment was carried out in triplicate.

Proximate and Fatty Acid Composition

Moisture (A.O.A.C., 1995), protein (A.O.A.C., 1995) and crude lipid (Folch, Lees, & Stanley, 1957) contents of rotifer and artemia were analyzed. Fatty acid methyl esters were obtained by transmethylation with 1% sulphuric acid in methanol (Christie, 1989). Fatty acid methyl esters were separated by GC (GC-14A; Shimadzu, Tokyo, Japan) using helium as a carrier gas. Fatty acid methyl esters were quantified by FID following the conditions (Izquierdo, Watanabe, Takeuchi, Arakawa, & Kitajima. 1990) and identified by comparison with external standards and well characterized fish oils (EPA 28, Nippai, Ltd Tokyo, Japan).

Statistical Analysis

All data were statistically treated using SPSS Statistical Software System 15.0 (SPSS, www.spss.com). The significant level for all the analysis was set 5%, and results are given as mean values and results standard deviation. Also, all variables were checked for normality and homogeneity of variance, using the Levene's tests respectively. To compare means, the group data were statistically tested using one-way ANOVA. When variances were not homogenate, a non-parametric Kruskal–Wallis test was accomplished.

Results

Rotifer Growth Performance

Egg number was found higher in rotifers fed with the S.parkle at day 3 (Figure 1a). After that day, egg number started to decrease in feeding experiment. At final day of the experiment, S.parkle and *Chlorella vulgaris* groups showed highest number of egg. Juvenile rotifer number was found higher in Beaker's yeast group then that in other groups (Figure 1b). Among the groups, S.parkle and Beaker's yeast showed the best egg production number. Egg carrying female number was decreased starting from feeding of day 2 (Figure 2c). Total biomass of rotifer was enhanced by feeding with S.parkle and Beaker's yeast at culture of day 6 (Figure 2d).

Biochemical Composition of Rotifer and Artemia

At the end of the study, crude protein content of rotifer was different among groups ($P<0.05$). Microalgae mixture enriched rotifers showed higher protein content than that in other groups. Lipid content was significantly higher in rotifer-enriched n-3 Top Rich group ($P<0.05$). Ash content was higher in Olio w-3 group rotifers and the lowest value was in rotifer enriched Emulsion T groups ($P<0.05$). Similarly, moisture content of rotifer enriched Emulsion T was found higher than Microalgae Mix, Red Pepper and Olio w-3 and n-3 Top Rich, respectively ($P<0.05$) (Table 1).

Crude protein content of artemia was not significantly ($P>0.05$) different among experimental groups. However, lipid content of artemia enriched Red Pepper showed higher value than other groups ($P<0.05$). Ash content of artemia was significantly higher in Emulsion T group than that in other groups ($P<0.05$). Moisture contents were significantly different among groups and the highest level was in W3 Olio group artemia ($P<0.05$) (Table 2).

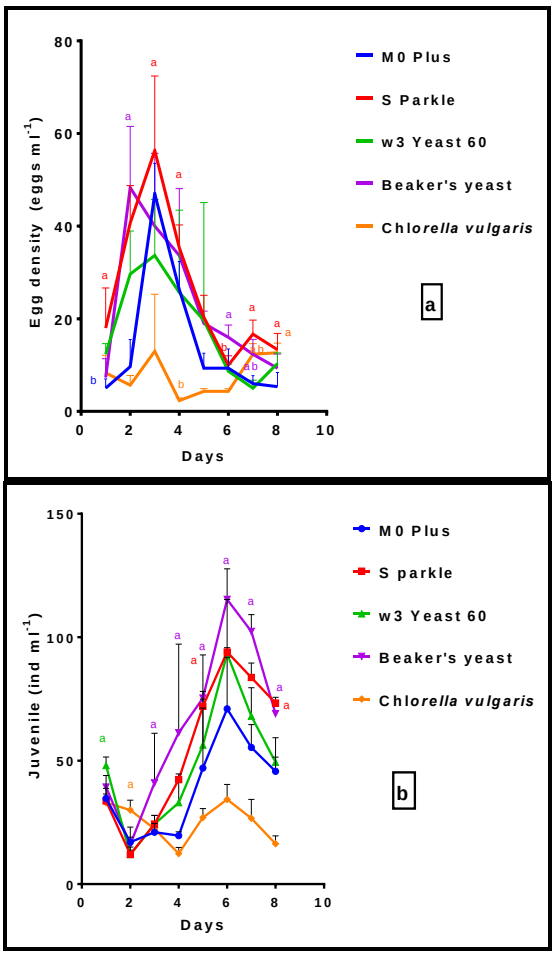

Figure 1. Egg (a) and juvenile number (b) of rotifer fed with different commercial feeds and microalgae (mean±SD, n=5). Values with the same superscript are not significantly different (P<0.05).

Fatty Acid Composition of Rotifer and Artemia

At the end of the enrichment, EPA (20:5n-3), DHA (22:6n-3), total n-3, total n-6, total n-3 HUFA levels of rotifer were enhanced by utilization of n-3 Top Rich product (P<0.05). Similarly, linoleic acid (LA; 18:2n-6) and linolenic acid (LNA; 18:3n-3) levels were higher in rotifer-enriched n-3 Top Rich. n-3/n-6 ratio was also higher in n-3 Top Rich group. However, ARA (20:4n-6) level of rotifers was supported by utilization of microalgae mixture (P<0.05). Oleic acid (18:1n-9) level was higher in rotifer enriched Emulsion T (Table 3). Similarly, oleic acid level was supported in artemia enriched Emulsion T and Microalgae mixture groups (P<0.05). Total n-3 HUFA level was higher in Red pepper group whereas EPA, DHA, total n-3 and total n-6 levels were higher in artemia enriched Culture Selco. ARA level of artemia was high in red pepper group (P<0.05) (Table 4).

Discussion

Live prey are much lower in certain essential fatty acids, amino acids, vitamins and minerals than copepods which are the natural prey of marine fish larvae (Lubzens, Zmora, & Barr, 2001; Hamre, Srivastava, Rønnestad, Mangor-Jensen, & Stoss, 2008; Hamre, 2016). Therefore, in order to get high survival in marine fish larvae, higher nutrient quality and quantity in rotifer and artemia should be maintained at larval period (Izquierdo & Koven, 2011). Enrichment process is one of the most important application in marine fish hatcheries. Enrichment products and methods (intervals and application time) play important role for the retention of essential nutrients in live prey and protocols differ among hatcheries (Furuita, Takeuchi, Toyota, & Watanabe, 1996). Despite the importance of nutritional value of live prey in development and survival of marine fish larvae, the nutritional effects of different feeds and enrichment products are still not optimized (Izquierdo, 1996; Izquierdo, Socorro, Arantzamendi, & Hernández-Cruz, 2000; Monroig, Navarro, Amat, Gonzalez, Bermejo, & Hontoria, 2006). Therefore, in this study, the effects of different rotifer feeds and enrichments on growth, biochemical composition and fatty acid profile of rotifer-artemia were investigated.

Rotifer numbers need to be increased rapidly

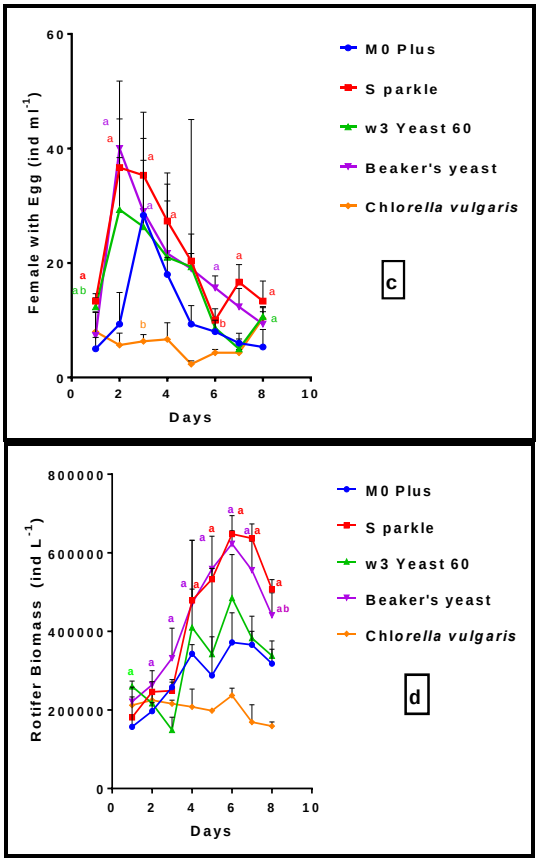

Figure 2. Egg carried female (c) and total rotifer biomass (d) number during feeding experiment. (mean±SD, n=5). Values with the same superscript are not significantly different (P<0.05).

Table 1. Proximate composition (g 100 g^{-1} dry weight) for whole body of rotifer biomass at the end of the enrichment experiment. Different letters within a line denote significant differences (P<0.05). Values expressed in mean ± SD (n =3 tanks/diet)

Rotifer	Control	n-3 Top Rich	Olio w3	Red Pepper	Microalgae Mix	Emulsion T
Crude protein	52.59±0.87	54.62±0.65^{d}	57.14±0.85^{c}	59.05±0.45^{b}	60.45±0.95^{a}	59.05±0.96^{b}
Crude lipids	8.96±0.06	18.38±0.99^{a}	10.25±0.58^{c}	13.25±0.88^{b}	10.21±0.32^{c}	10.52±0.89^{c}
Ash	1.62±0.31	1.03±0.66^{b}	1.32±0.45^{a}	0.93±0.22^{c}	1.11±0.17^{b}	0.86±0.44^{d}
Moisture	9.65±0.37	9.33±0.24^{d}	8.85±0.45^{e}	9.70±0.85^{c}	10.22±0.54^{b}	10.87±0.65^{a}

Table 2. Proximate composition (g 100 g^{-1} dry weight) for whole body of artemia at the end of the enrichment experiment. Different letters within a line denote significant differences (P <0.05). Values expressed in mean ± SD (n =3 tanks/diet)

Artemia	Control	n-3 TOP RICH	W3 Olio	Red Pepper	Microalgae Mix	Emulsion T	Selco
Crude Protein	4.67±0.28	5.24 ±1.46^{a}	6.44±0.66^{a}	5.60±0.29^{a}	5.64±0.51^{a}	5.75±0.22^{a}	5.44±0.65^{a}
Crude Lipid	0.45±0.22	0.69±0.01^{d}	0.79±0.02^{cd}	1.31±0.06^{a}	0.92±0.05^{b}	0.89±0.04^{bc}	0.72±0.04^{d}
Crude Ash	0.72±0.01	0.50±0.14^{ab}	0.54±0.19^{ab}	0.51±0.03^{ab}	0.25±0.02^{c}	0.34±0.43^{a}	0.56±0.32^{bc}
Moisture	6.05±0.21	7.91±0.26^{a}	9.67±0.52^{b}	7.39±1.49^{b}	6.37±1.22^{c}	7.98±0.49^{b}	7.56±0.26^{b}

during fish larval production period. At this point, physical, chemical and nutritional factors play important role (Lubzens, Zmora, & Barr, 2001). Nowadays, not only commercial rotifer feeds but also freshly cultured microalgae are being evaluated for rotifer cultivation. Özbaş, Göksan, & Ak (2006) have reported that total rotifer and egg carrying female rotifer numbers were continuously increased by feeding fresh microalgae (*Nannochloropsis* sp.). Similarly, Nhu (2004) has mentioned about the beneficial effects of fresh *Nannochloropsis oculata* on maximum growth rate of rotifer. In another study, freshly cultured *Pavlova viridis* significantly increased rotifer and egg numbers whereas egg ratio was enhanced by Beaker's yeast fed rotifers in laboratory scale experiment (Rehberg-Haas, Meyer,

Tablo 3. The fatty acid compositions of rotifers (*Brachionus plicatilis*) enriched twice a day and after 24h of enrichments (% total fatty acids)

	Unenriched	n-3 TOP RICH	Olio w3	Red Pepper	Microalgae Mix	Emulsion T
4:0	-	0.16±0.03	-	-	-	-
10:0	-	0.24±0.01	-	0.01±0.01	-	0.01±0.00
12:0	-	0.77±0.04^{a}	0.25±0.01^{c}	0.45±0.01^{b}	0.72±0.00^{a}	0.07±0.00^{d}
13:0	-	0.03±0.01	-	-	-	0.03±0.01
14:0	2.190	3.60±0.11^{d}	5.67±0.02^{b}	9.52±0.09^{a}	3.47±0.01^{d}	4.68±0.01^{c}
14:1	-	0.11±0.02	0.17±0.01	-	-	0.03±0.00
15:0	0.630	0.45±0.01^{c}	0.72±0.01^{b}	0.87±0.02^{a}	0.35±0.00^{d}	0.35±0.01^{d}
16:0	13.240	12.60±0.07^{d}	29.74±0.13^{b}	35.66±0.76^{a}	15.91±0.05^{c}	15.36±0.25^{c}
16:1	-	6.95±0.08^{a}	7.03±0.08^{a}	7.06±0.37^{a}	2.60±0.01^{c}	4.97±0.02^{b}
17:0	0.520	0.40±0.01	1.25±0.01	0.51±0.01	-	0.30±0.02
18:0	4.010	3.98±0.01^{c}	16.90±0.04^{a}	-	4.56±0.01^{b}	-
18:1n-9	13.700	12.36±0.05^{d}	23.45±0.20^{b}	16.23±0.16^{c}	10.48±0.04^{e}	45.55±0.13^{a}
18:2n-6	7.670	7.09±0.18^{a}	1.28±0.02^{e}	3.11±0.23^{c}	1.95±0.01^{d}	6.03±0.03^{b}
18:3n-3	1.950	1.76±0.07^{a}	0.15±0.06^{d}	0.77±0.11^{c}	0.35±0.01^{d}	1.19±0.01^{b}
18:3n-6	0.210	0.10±0.01	-	-	-	0.01±0.00
20:0	0.090	0.14±0.06	0.24±0.01	0.33±0.01	-	0.34±0.00
20:1n-9	1.640	2.81±0.02^{b}	1.16±0.08^{d}	2.19±0.13^{c}	-	4.58±0.04^{a}
20:3n-3	0.001	0.50±0.01^{b}	0.21±0.03^{c}	0.50±0.00^{b}	-	0.64±0.00^{a}
20:3n-6	0.250	0.14±0.06^{b}	-	0.44±0.09^{a}	-	0.03±0.00^{c}
20:4n-6	1.500	-	0.08±0.00^{c}	0.81±0.06^{b}	1.40±0.01^{a}	0.04±0.00^{c}
20:5n-3	0.007	10.20±0.08^{a}	-	0.35±0.01^{b}	-	0.07±0.00^{c}
22:0	-	-	-	0.20±0.01	-	0.14±0.00
22:2	-	-	-	0.06±0.01	-	0.08±0.00
22:5n-3	6.960	1.86±0.04	-	-	-	0.08±0.00
22:6n-3	9.030	16.84±1.16^{a}	-	1.22±0.04^{b}	-	0.05±0.00^{c}
23:0	-	-	-	0.26±0.00	-	0.03±0.01
24:0	0.070	0.07±0.01	-	0.18±0.01	-	0.05±0.01
24:1	0.015	0.06±0.04	0.16±0.01	0.36±0.00	-	0.31±0.01
Σ Saturated	20.680	22.42±0.30^{d}	54.81±0.21^{a}	48.04±0.66^{b}	25.01±0.08^{c}	21.34±0.23^{e}
Σ Monounsaturated	40.920	7.66±0.06^{a}	7.36±0.08^{a}	7.42±0.37^{a}	2.60±0.01^{c}	5.30±0.03^{b}
Σ n-3	22.021	31.16±1.22^{a}	0.36±0.08^{c}	2.84±0.13^{b}	0.35±0.01^{c}	2.03±0.01^{b}
Σ n-6	9.782	7.33±0.23^{a}	1.36±0.02^{e}	4.35±0.39^{c}	3.34±0.01^{d}	6.11±0.03^{b}
Σ n-9	19.200	15.16±0.03^{d}	24.61±0.12^{b}	18.42±0.04^{c}	10.48±0.04^{e}	50.12±0.17^{a}
Σ n-3 HUFA	18.461	29.40±1.29^{a}	0.36±0.08^{b}	2.07±0.03^{b}	-	0.84±0.00^{b}
EPA/ARA	4.640	-	-	0.43±0.02	-	1.75±0.00
DHA/EPA	1.297	1.65±0.10	-	3.49±0.26	-	0.71±0.00
DHA/ARA	6.020	-	-	1.52±0.17	-	1.25±0.00
n-3/n-6	2.251	4.25±0.30^{a}	0.27±0.06^{c}	0.65±0.09^{b}	0.10±0.00^{c}	0.33±0.00^{bc}

Lippemeier, & Schulz, 2015). At the same study, large scale rotifer cultivation was investigated by several microalgae feeds and *Nannochloropsis* sp. utilization resulted better rotifer culture performance (Rehberg-Haas, Meyer, Lippemeier, & Schulz, 2015). *Chlorella vulgaris* has been noted as a suitable feed for *Proale similis* (Lee, Kim, Lee, Hagiwara, Kwon, Park, & Park, 2016). Despite *Chlorella vulgaris* has higher biomass potential, protein and B_{12} content in comparison to *Nannochloropsis oculata* (Maruyama, Nakao, Shigeno, Ando, & Hirayama, 1997), the utilization of freshly cultured *Chlorella vulgaris* did not well perform in laboratory scale rotifer culture in our study. In fact, the highest total rotifer number was increased by feeding Beaker's yeast and S.parkle products. In agreement with our findings, Lind (2014) reported the highest rotifer growth in those fed S.parkle and Reed Rotigrow *Nannochloropsis*. Moreover, S.parkle substitution by Beaker's yeast with different proportions has not resulted in significant differences in terms of growth. In our study, one of the reason of insignificant effect of *Chlorella vulgaris* on rotifer growth performance could be related to low level of B_{12} as Hirayama, Maruyama, & Maeda (1989) reported. However, Vibrio concentration increased when rotifer fed 75% Beaker's yeast substituted diet. These results could be related to vitamin B_{12} and protein content of *Saccharomyces cerevisiae* as previously reported in other studies (Lubzens, Tandler, & Minkoff, 1989; Ferreira, Pinho, Vieira, & Tavarela, 2010). Conversely, different forms of yeast have positive

Table 4. The fatty acid compositions of artemia enriched twice a day and after 24h of enrichments (% total fatty acids)

	Unenriched	n-3 TOP RICH	W3 Olio	Red Pepper	Microalgae Mix	Emulsion T	Selco
4:0	0.16±0.00	-	-	-	-	-	-
10:0	0.13±0.00	-	0.52±0.06	0.10±0.01	0.37±0.01	-	-
12:0	0.26±0.01	0.28±0.04^{d}	0.72±0.01^{b}	0.22±0.01^{d}	0.85±0.01^{a}	0.16±0.05^{e}	0.39±0.03^{c}
13:0	0.04±0.00	-	-	-	-	-	-
14:0	2.67±0.02	2.02±0.00^{e}	4.28±0.06^{a}	3.52±0.04^{b}	3.28±0.00^{c}	2.10±0.01^{e}	2.62±0.06^{d}
14:1	-	-	-	0.22±0.02	-	-	-
15:0	0.54±0.00	0.38±0.00^{b}	0.48±0.04^{a}	0.49±0.02^{a}	0.34±0.01^{bc}	0.23±0.03^{d}	0.25±0.01^{cd}
16:0	30.97±0.05	26.68±0.04^{c}	24.37±0.16^{c}	24.09±0.17^{c}	27.17±0.04^{b}	33.08±0.27^{a}	18.43±0.19^{d}
16:1	3.67±0.06	0.66±0.00^{c}	3.42±0.27^{b}	3.74±0.09^{b}	3.16±0.04^{b}	3.34±0.25^{b}	4.69±0.01^{a}
17:0	1.05±0.01	1.39±0.01^{a}	0.70±0.03^{b}	0.83±0.00^{b}	0.72±0.01^{b}	0.84±0.06^{b}	0.49±0.06^{c}
18:0	6.89±0.08	16.65±0.02^{a}	8.35±0.30^{e}	11.53±0.08^{b}	10.80±0.03^{c}	8.94±0.01^{d}	6.89±0.10^{f}
18:1n-9	20.66±0.22	12.39±0.02^{c}	20.36±0.66^{b}	21.16±0.45^{b}	26.23±0.16^{a}	25.72±0.20^{a}	21.15±0.43^{b}
18:2n-6	1.44±0.03	2.05±0.01^{d}	3.55±0.06^{c}	5.14±0.08^{b}	3.50±0.01^{c}	0.44±0.02^{e}	7.63±0.03^{a}
18:3n-3	5.31±0.25	2.07±0.00^{e}	10.66±0.40^{b}	9.19±0.06^{c}	4.24±0.01^{d}	0.21±0.06^{f}	13.55±0.12^{a}
18:3n-6	0.02±0.00	-	0.12±0.02	-	-	-	-
20:0	0.12±0.00	-	-	0.19±0.00^{c}	0.33±0.01^{b}	0.18±0.04^{c}	0.53±0.06^{a}
20:2	-	-	-	-	-	-	-
20:1n-9	0.32±0.00	-	0.48±0.08^{ab}	0.36±0.01^{c}	0.54±0.01^{b}	0.49±0.01^{ab}	0.92±0.02^{a}
20:3n-3	-	-	-	-	-	-	-
20:3n-6	-	-	-	-	-	-	-
20:4n-6	0.06±0.00	-	0.37±0.00^{c}	0.66±0.01^{a}	-	-	0.53±0.06^{b}
20:5n-3	0.16±0.01	-	-	1.24±0.02^{b}	-	-	3.43±0.06^{a}
21:0	0.19±0.01	2.12±0.00	-	-	-	-	-
22:0	0.12±0.00	-	0.38±0.05^{b}	0.18±0.01^{c}	0.66±0.00^{a}	0.19±0.02^{c}	0.39±0.02^{b}
22:2	0.31±0.02	2.51±0.01	-	-	-	-	-
22:5n-3	-	-	-	0.07±0.01^{b}	-	-	0.55±0.03^{a}
22:6n-3	-	-	1.00±0.05^{b}	2.60±0.01^{a}	-	-	2.49±0.04^{a}
23:0	0.08±0.01	-	-	0.83±0.01	0.42±0.01	-	0.50±0.03
24:0	-	-	-	0.04±0.01	-	-	-
24:1	-	-	-	-	-	-	-
Σ Saturated	43.20±0.06	47.51±0.11^{a}	39.79±0.23^{d}	41.55±0.23^{c}	44.92±0.03^{b}	45.70±0.27^{b}	30.47±0.31^{e}
Σ Monounsaturated	3.67±0.06	0.66±0.00^{d}	3.42±0.27^{bc}	3.95±0.11^{b}	3.16±0.04^{c}	3.34±0.25^{bc}	4.69±0.01^{a}
Σ n-3	5.47±0.26	2.07±0.00^{e}	11.65±0.45^{c}	13.09±0.06^{b}	4.24±0.01^{d}	-	20.02±0.16^{a}
Σ n-6	1.52±0.03	2.05±0.01^{e}	4.03±0.04^{c}	5.79±0.08^{b}	3.50±0.01^{d}	0.44±0.02^{f}	8.16±0.09^{a}
Σ n-9	20.98±0.22	12.39±0.02^{d}	20.84±0.59^{c}	21.52±0.46^{bc}	26.76±0.17^{a}	26.21±0.21^{a}	22.06±0.45^{b}
Σ n-3 HUFA	0.16±0.01	-	1.00±0.05^{c}	13.09±0.06^{a}	-	-	6.47±0.04^{b}
EPA/ARA	2.58±0.12	-	-	1.89±0.05^{b}	-	-	6.53±0.91^{a}
DHA/EPA	-	-	-	2.11±0.05^{a}	-	-	0.73±0.02^{b}
DHA/ARA	-	-	2.89±0.08^{c}	3.97±0.02^{b}	-	-	4.74±0.50^{a}
n-3/n-6	3.60±0.11	1.01±0.00^{d}	-	2.26±0.04^{b}	1.21±0.01^{c}	-	2.45±0.05^{a}

effect on rotifer growth. For instance, Nhu (2004) also obtained maximum density of rotifer when fed with wet yeast feed. Not only vitamins but also other micronutrients are also important for rotifer culture performance (Yoshimatsu, Higuchi, Zhang, Fortes, Tanaka, & Yoshimura, 2006).

Fatty acid composition of rotifer and artemia were improved by enrichment products in our study. As a primary energy source, oleic acid level of rotifers was fortified by Emulsion T enrichment in Experiment II. In fact, oleic acid is a primer energy source for fish larvae and it is a very important fatty acid for larval energy budget especially at first exogenous feeding period (Cahu & Infante, 2001; Yufera & Darias, 2007). Therefore, a content of higher oleic acid level is desirable in live prey (Izquierdo, 1996). Total n-3 level of rotifer was supported by n-3 Top Rich product. However, ARA level was significantly higher in rotifer enriched microalgae mixture. Therefore, the combined usage of a couple of enrichers in live prey seem to be a good option in order to maintain optimum essential fatty acid ratio. Based on our results, n-3 Top Rich and freshly cultured microalgae mixture is highly

recommendable for rotifer enrichment.

As for artemia, enrichment with Culture Selco seemed to be the best option for improving the nutritional value of artemia whereas ARA level was enhanced by the utilization of Red Pepper. ARA is a very important fatty acid that takes action in survival, immune metabolism and stress resistance in marine fish (Bell & Sargent, 2003). Han, Geurden, & Sorgeloos (2001) have argued that when artemia enriched emulsions containing essential fatty acids with different proportions such as DHA/Oleic acid, EPA/Oleic acid and AA/Oleic acid resulted in different retention of fatty acids. Among them EPA and oleic acid were the most efficiently remained fatty acids in artemia at the end of the 24 hours enrichment. To sum up, the combined usage of more than one enrichment for artemia could be a good the best option, such as Red Pepper and Culture Selco.

In this study, we specifically focused on culture performance of rotifer and fatty acids profile of enriched rotifer and artemia. Baker's yeast *Saccharomyces cerevisiae* is still cheap feed source and seemed to be performed efficiently in rotifer culture. Nevertheless, further experimental studies on feeding by alternative yeast products in rotifer cultivation are necessary. In terms of enrichment process, EPA and DHA levels were supported by n-3 Top Rich product in rotifer. However, ARA level of rotifer was increased by the utilization of fresh microalgae mixture. Culture Selco is strongly adviced for nutritional boosting of artemia. The beneficial support of the commercial products in terms of fatty acid composition and vitamins (C and E) was also suggested in Hamre (2016). Recently, micronutrients such as iodine, selenium, copper and manganese have become more important for enrichment process (Penglase, Hamre, Sweetman, & Nordgreen, 2011; Ribeiro, Ribeiro, Dinis, & Moren, 2011; Srivastava, Hamre, Stoss, & Nordgreen, 2012; Nordgreen, Penglase, & Hamre, 2013). Delivering micronutrients to rotifers can be managed by enriching microalgae (Doucha, Livansky, Kotrbacek, & Zachleder, 2009; Kouba, Velíšek, Stará, Masojídek, & Kozák, 2014). This could be an edequate solution if nutritional value of microalgae could be increased. Further studies are needed for investigation into mineral composition in enrichments and retention in live prey in feeding and enrichment of live prey in Turkey.

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