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Full Length Research Paper

Effects of commercial enrichment products on fatty acid components of rotifer, *Brachionus plicatilis*

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This study was undertaken to test the effects of enrichment products. Red pepper paste (ZA), AlgaMac 3050 (ZB) and Spresso (ZC) on fatty acid compositions in rotifers (*Brachionus plicatilis*) which were intensively cultured on a mixture of *ω*3 algae and *ω*3 yeast. Enriched rotifers were seen to have higher level of unsaturated fatty acids of linoleic acid (LOA) and total n-6 unsaturated fatty acid ratios than all other experimental groups ($p < 0.05$). The levels of arachidonic acid (ARA) in rotifers groups of ZB and ZC were different from all the remaining groups ($p < 0.05$), but it was high in starved rotifers. The levels of docosapentaenoic acid (n–6 DPA) and total n–3 multiple unsaturated fatty acids, the level of docosahexaenoic acid (DHA) and the ratio of n–3/n–6 in enriched rotifers groups were higher ($p < 0.05$). The level of α-linolenic (αLNA) in ZA rotifer group was lower than other rotifer groups ($p < 0.05$) and the amount of eicosadienoic acid (EPA) was the same for all groups. Highest ratios of n–6DPA/ARA, DHA/EPA and DHA/ARA were obtained from ZC rotifer group ($p < 0.05$). Similar ratios of DHA/n–6DPA were observed for all groups ($p > 0.05$). The ratio of n–6DPA/ARA was different between the groups of ZA and ZC ($p < 0.05$). Significant differences between ZA, ZB and ZC rotifer groups were observed in the ratio of n–3/n–6, DHA/EPA, DHA/ARA and n–6DPA. The differences in EPA/ARA ratios between ZB and ZC rotifer groups were significant from other groups ($p < 0.05$). It can be suggested that the enrichment of rotifers by available commercial products lead to significant and important changes in fatty acid profiles which are of high concern in the quality of nutrient supply for improved productivity.

Key words: Rotifers, *Brachionus plicatilis*, enrichment, fatty acids.

INTRODUCTION

The use of rotifers has been recommended as they stimulate group of larvae and improve survival rate as well as their potentiality of improved nutrient content by commercial enrichment products (Sargent et al., 1997; Conceição et al., 2010). Rotifers contain appreciable amount of enzymes which helps the larvae to develop digestive system and moreover ratifers can homo-genously be distributed in the tanks and can be used as live-feed capsules (Gamsız, 2002; Lubzens and Zmora, 2003). Aquatic organisms are not able to synthesize docosahexaenoic acid (DHA), eicosadienoic acid (EPA), arachidonic acid (ARA), linolenic acid (LNA) and linoleic acid (LOA), and these fatty acids are essential to be exogenously provided (Glencross, 2009).

Rotifers are reported to catabolize fats easily and can store highly unsaturated fatty acids (HUFA) (Haché and Plante, 2011). Rotifers fed on Baker's yeast lacked sufficient amount of DHA, EPA and ARA (Olsen et al., 2004). Significant changes were observed in the profiles of n–3HUFA, n–6PUFA and total lipids in the rotifers groups fed on various lipid emulsifying solutions (Fernández–Reiriz et al., 1993; Rodríguez et al., 1996, 1997; Øie and Olsen, 1997; Pousão-Ferreira et al., 1997; Blair, 2005).

Rotifers were enriched on three different commercial products containing various levels of protein (P), lipid (L) and P:L (N) ratios by Øie et al. (1997). L and P group rotifers had significantly high level of total fatty acid levels than the group of N rotifers ($p < 0.05$). The contents of EPA, DHA and n–3HUFA were high in L rotifer group and low in P group rotifers ($p < 0.05$). Ratio of DHA/EPA in L

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rotifer group was higher than the groups of N and P rotifers.

In comparison to the rotifers fed on algae, the rotifers fed on commercial enrichment products had increased levels of linoleic acid, α-linolenik, ARA, DHA, EPA, n–6HUFA, n–3DPA, ΣHUFA, n–3HUFA and DHA/EPA, Σω3/Σω6 (Seiffert et al., 2001; MacDonald, 2004; Faulk et al., 2005; Cavalin and Weirich, 2009). Various profiles of fatty acids such as the contents of ΣSFA, ΣMUFA, ARA, EPA, DHA ΣPUFA, total ω3, ω6, ω3/ω6, DHA/EPA, EPA/ARA and DHA/DPA, ω6DPA/ARA and ω6DPA in rotifers were significantly changed by similar commercial enrichment products (O'Brien–MacDonald et al., 2006; Garcia, 2006; Garcia et al., 2008a,b,c; Naz, 2008; Westelmajer, 2008; Kotani et al., 2009; Roo et al., 2009, 2010a, b; Copeman, 2001).

Despite the fact that the production of live-feed can be increased by enrichment and recent culturing techniques, the quality of such feeds in terms of nutritive value are not always reliable (Naz, 2008). Early larvae development and improved survival rate in seafish production can be manageable by the application of enrichment of live-feed sources in hatcheries.

The objective of this study was to apply three commercial enrichment products for the production of rotifers feeds according to the manufacturer's recommendation for doses and application period and to analyse the fatty acid profiles.

MATERIALS AND METHODS

Rotifers (*B. plicatilis* S-strain, Egemar Su Ürünleri Gıda ve Sanayi ve Ticaret AŞ, Aydın Turkey) were reared for 72 h in 2000-L conical tanks (V-type) at 24 h artificial illumination with continuous oxygen supply. In this study, sea water was used and was filtered through sand, cartridge, ultraviolet and biological filters and regulated salinity 0.25%, temperature 27 ± 0.50°C, dissolved oxygen 8.3 to 14.6 mg/L and pH 7.4 ± 0.5.

Rotifers cultures

The mass rotifers cultures (K) were fed with frozen mikroalgae of ω3 Algae (BernAqua NV Hagelberg 3 B–2250 Olen Belgium) (K-1) and powdered (*S. Cerevisiae*) ω3 Yeast 60® (BernAqua NV Hagelberg 3 B–2250 Olen Belgium) (K–2). During this period, a group (T) was fed on a mixture containing 25% of K-1 and 75% of K-2 every 4 h per day. This mixture was then fed twice a day on a dose of 0.6 g for 0 to 23 h (T_0), 0.5 g for 24 to 47 h (T_1) and 0.4 g/million rotifers for 48 to 71 h (T_2). Initially, 350 rotifers per ml was cultured and during the experimental period, the rotifers counts were made in the mornings and afternoons. When the count reached 500 rotifers per ml at the end of T_0 and 1000 rotifers per ml at the end of T_2, harvesting began at 72 h (T_3).

Rotifers enrichment

Enrichment (Z) was performed on the cultured rotifiers using three commercial products according to the recommended doses and periods. This study was done at a commercial scale. Rotifers were stocked in 400-L conical tanks (V-type) at room temperature under 24 h artificial illumination in a seawater, filtered through cartridge, UV and biologic filters with 0.25% salinity, a temparature of 26.0 to 26.5°C, a pH of 7.4 ± 0.5 and a 19.0 ± 1 mg/L oxygen (O_2).

The enrichment treatments were as follows: The rotifers enriched with red pepper paste (BernAqua NV Hagelberg 3 B–2250 Olen Belgium) (ZA) were stocked as 500 per ml (196 million per 400 L) and fed on a dose of 180 g/m^3 for 6 h and were then harvested.

The rotifers enriched with AlgaMac 3050 (Aquafauna Bio–Marine Inc. PO Box 5 Hawthorne, California USA) (ZB) were stocked as 1000 per ml (194 million per 150 L) and fed on a dose of 0.3 g/m^3 for 8 h and were then harvested.

The rotifers enriched with Spresso (Inve Aquaculture nv Hoogveld 91 9200 Dendermonde Belgium) (ZC) were stocked as 1000 per ml (195 million per 150 L) and fed on a dose of 175 g/m^3 for 12 h, and were then harvested. Furthermore, the group K rotifies (0 time) were stocked at 600 L around 597 millions and were then starved (Z0) in 600 L around 604 millions.

Rotifers were enriched twice a day, based on the manufacturer's recommendations and on the technical feasibility of a semi-commercial hatchery operation.

Rotifers were harvested after 24 h on a 60 µm filter and gently rinsed with filtered seawater for 5 min before sampling for nutritional analysis. All samples were made in triplicate. In addition, the samples were obtained from the rotifer group fed on a mixture of feed containing K-1 + K-2 at 6 h (ZKA), at 8 h (ZKB) and at 12 h (ZKC), similar time scales of the groups of ZA, ZB and ZC, respectively. All treatments mentioned above K (control), K_0 (starved control), ZA, ZKA (positive control), Z0A (negative control-starved), ZB, ZKB, Z0B, ZC, ZKC and Z0C were tested together.

Chemıcal and statıstıcal analyses

Fatty acid analysis was done on the wet weight bais of three replicates. A direct methylation was applied before the extraction of rotifer fats. A 15 ml glass tubes with screw was poured with a sample of 0.2 to 0.5 g and added with 1.5 ml methanolic NaOH. The tubes were left on 115°C for 7 min, and this was followed by cooling at 45°C. Later the contents were added with 2 ml methanolic borontriflorid (BF3) and left again at 115°C for 5 min, before it was cooled down. Afterward, a 2 ml of izo–octan and saturated 3 ml of NaCl solution were added and shaken for 1 min. Separation of phases was done after 2 min. Organic phase was separated by centrifugation at 1000 rpm for 1 min. 1.5 ml of supernatant was transferred to glass vials and stored at -20°C before analysis. Gas chromatographic analysis (GC) was done in a split mode of 1:20 using Hewlett Packard 6890 enjektor of 1 µl (Öksüz and Özyılmaz, 2010). Fatty acids were analysed by GC with MS detector. Injection and detector temperatures were 250 and 270°C. Split ratio was 1:20. Separation of fatty acids was done within 20 min, using standards of FAME (Supelco 47085U PUFA No: 3) compared on FAME (Supelco 47885–U).

Analysis of variance using Windows SPSS 15.0 program was employed to see any significant effects of treatments and the differences between the group means were separated at a probability of 0.05 by Tukey' test (Eymen, 2007).

RESULTS

The chromatography of GS–MS provided approximately 29 fatty acids in rotifer groups (Table 1). Highest fatty acid content was obtained from ZA group of rotifers enriched on red pepper paste (99.00 ± 0.55%) and lowest value from ZOB rotifer group (96.17 ± 0.24%) (Table 1).

Table 1. Fatty acid compositions of rotifers, enriched on commercial products (mean ± standard error %).

YA	K	ZA	ZKA	Z0A	ZB	ZKB	Z0B	ZC	ZKC	Z0C
12:0	-	0.05 ± 0.05^{a}	-	-	0.35 ± 0.35^{a}	-	-	-	-	-
14:0	2,93 ± 0.07^{ef}	6.86 ± 0.11^{b}	3.33 ± 0.09^{de}	2.77 ± 0.03^{f}	8.45 ± 0.12^{a}	3.32 ± 0.10d^{e}	2.87 ± 0.04^{f}	4.27 ± 0.05^{c}	3.47 ± 0.02^{d}	2.78 ± 0.10^{f}
15:0	1.14 ± 0.06^{a}	0.57 ± 0.08^{d}	0.74 ± 0.04^{cd}	1.16 ± 0.02^{a}	-	0.86 ± 0.03^{bc}	1.15 ± 0.02^{a}	0.37 ± 0.01^{e}	1.04 ± 0.01^{ab}	1.14 ± 0.01^{a}
16:0	19.10 ± 0.39^{e}	26.39 ± 0.54^{a}	18.04 ± 0.25^{ef}	17.73 ± 0.06^{ef}	21.17 ± 0.17^{bc}	18.48 ± 0.48^{ef}	17.37 ± 0.22^{f}	22.46 ± 0.26^{b}	20.78 ± 0.24^{d}	17.51 ± 0.29^{ef}
17:0	0.55 ± 0.03^{abc}	0.11 ± 0.10b^{c}	0.29 ± 0.14^{abc}	0.86 ± 0.02^{a}	-	0.35 ± 0.19^{abc}	0.39 ± 0.20^{abc}	0.34 ± 0.01^{abc}	0.32 ± 0.16^{abc}	0.63 ± 0.03^{ab}
18:0	5.50 ± 0.08^{b}	3.23 ± 0.12^{d}	4.58 ± 0.09^{c}	5.74 ± 0.15^{ab}	2.99 ± 0.14^{d}	4.77 ± 0.16^{c}	5.60 ± 0.09^{b}	3.23 ± 0.02^{d}	5.58 ± 0.02^{b}	6.29 ± 0.19^{a}
20:0	-	-	-	-	-	-	-	0,15 ± 0,00^{a}	-	-
ΣSFA	29.22 ± 0.31^{cd}	37.21 ± 0.68^{a}	26.98 ± 0.39^{e}	28.26 ± 0.19d^{e}	32.96 ± 0.26^{b}	27.78 ± 0.77^{de}	27.38 ± 0.24^{de}	30.80 ± 0.30^{c}	31.19 ± 0.14b^{c}	28.35 ± 0.25^{de}
16:1n-7	6.61 ± 0.08^{cd}	4.08 ± 0.04^{e}	7.64 ± 0.16^{a}	6.42 ± 0.08^{d}	2.67 ± 0.01^{f}	7.26 ± 0.26^{ab}	6.52 ± 0.07^{cd}	2.97 ± 0.09^{f}	7.02 ± 0.00^{bc}	6.51 ± 0.15^{cd}
16:1n-5	0.70 ± 0.02^{ab}	0.43 ± 0.43^{ab}	0.59 ± 0.01^{ab}	0.72 ± 0.02^{a}	-	0.60 ± 0.02^{ab}	0.74 ± 0.03^{a}	0.13 ± 0.13^{ab}	0.66 ± 0.00^{ab}	0.72 ± 0.03^{a}
17:1	-	-	-	1.97 ± 0.08^{a}	-	-	-	0.58 ± 0.01^{b}	-	-
18:1n-11c	2.80 ± 0.01^{c}	1.45 ± 0.06^{e}	1.92 ± 0.02^{d}	3.65 ± 0.04^{ab}	-	2.07 ± 0.05^{d}	3.44 ± 0.05^{b}	0.49 ± 0.24^{f}	2.63 ± 0.03^{c}	3.96 ± 0.07^{a}
18:1n-9c(OLA)	7.29 ± 0.32d^{e}	4.26 ± 0.14^{f}	10.14 ± 1.13^{abc}	7.97 ± 0.10^{cde}	5.30 ± 0.73^{ef}	11.02 ± 0.28^{ab}	9.78 ± 0.98^{abcd}	12.25 ± 0.36^{a}	8.67 ± 0.01^{bcd}	8.30 ± 0.18^{bcd}
18:1n-7c	2.57 ± 0.08^{a}	1.67 ± 0.05^{ab}	0.68 ± 0.68^{b}	2.53 ± 0.03^{a}	-	-	0.81 ± 0.81^{b}	-	2.55 ± 0.04^{a}	2.62 ± 0.00^{a}
20:1n-9	2.94 ± 0.11^{b}	1.57 ± 0.03^{d}	2.42 ± 0.10^{c}	3.84 ± 0.16^{a}	-	2.68 ± 0.03^{bc}	3.80 ± 0.08^{a}	1.76 ± 0.03^{d}	2.92 ± 0.01^{b}	4.11 ± 0.10^{a}
22:1n-9	-	-	1.01 ± 0.21^{a}	-	-	-	0.67 ± 0.67^{a}	0.00 ± 0.00	1.01 ± 0.13^{a}	-
ΣMUFA	2.91 ± 0.62^{d}	13.46 ± 0.24^{f}	24.40 ± 0.58^{bcd}	27.10 ± 0.18^{a}	7.97 ± 0.73^{g}	23.63 ± 0.64^{cd}	25.76 ± 0.46^{abc}	18.18 ± 0.16^{e}	25.46 ± 0.05^{c}	26.24 ± 0.13^{ab}
16:2n-4	2.02 ± 0.06^{c}	0.74 ± 0.01^{d}	2.72 ± 0.05^{a}	-	-	2.46 ± 0.05^{b}	1.96 ± 0.02^{c}	0.35 ± 0.01^{e}	2.03 ± 0.00^{c}	1.90 ± 0.01^{c}
16:4n-1	1.32 ± 0.07^{ab}	0.34 ± 0.17^{cd}	0.32 ± 0.16^{cd}	1.27 ± 0.20^{ab}	-	0.20 ± 0.20^{cd}	0.60 ± 0.30^{bcd}	0.12 ± 0.06^{cd}	0.89 ± 0.02^{abc}	1.53 ± 0.08^{a}
18:2n-6c(LOA)	16.24 ± 0.66^{bcd}	9.60 ± 0.27^{f}	15.62 ± 0.14^{cde}	18.05 ± 0.33^{a}	5.83 ± 0.21^{g}	15.26 ± 0.50^{de}	16.99 ± 0.23^{abc}	10.97 ± 0.13^{f}	14.50 ± 0.05^{e}	17.55 ± 0.13^{ab}
18:3n-6(γLNA)	-	0.07 ± 0.07^{a}	-	-	-	-	-	0.11 ± 0.06^{a}	-	-
20:2n-6	1.19 ± 0.11^{b}	0.84 ± 0.03^{c}	1.43 ± 0.04^{b}	1.32 ± 0.02^{b}	-	1.81 ± 0.03^{a}	1.34 ± 0.06^{b}	1.18 ± 0.15^{b}	1.41 ± 0.00^{b}	1.34 ± 0.04^{b}
20:3n-6	1.30 ± 0.01a	0.83 ± 0.01^{bc}	1.09 ± 0.03^{ab}	1.46 ± 0.01^{a}	0.26 ± 0.26^{d}	1.19 ± 0.08^{ab}	1.34 ± 0.01^{a}	0.60 ± 0.03^{cd}	1.10 ± 0.01^{ab}	1.39 ± 0.03^{a}
20:4n-6(ARA)	3.86 ± 0.14^{ab}	3.50 ± 0.03^{bc}	3.05 ± 0.12^{de}	4.10 ± 0.07^{a}	2.73 ± 0.12^{ef}	3.15 ± 0.13^{cde}	3.83 ± 0.02^{ab}	2.44 ± 0.03^{f}	3.24 ± 0.03^{cd}	3.80 ± 0.06^{ab}
22:5n-6(DPA)	3.35 ± 0.29^{de}	6.68 ± 0.07^{b}	4.49 ± 0.14^{c}	2.60 ± 0.08^{f}	11.12 ± 0,03^{a}	4.84 ± 0.15^{c}	2.71 ± 0.08^{ef}	6.79 ± 0.11^{b}	3.66 ± 0.04^{d}	2.19 ± 0.09^{f}
Σn-6PUFA	25.94 ± 0.43^{b}	21.52 ± 0.25^{d}	25.68 ± 0.16^{b}	27.53 ± 0.33^{a}	19.94 ± 0.30^{e}	26.25 ± 0.20^{ab}	26.21 ± 0.26^{b}	22.09 ± 0.11^{d}	23.91 ± 0.02^{c}	26.27 ± 0.26^{ab}
18:3n-3(αLNA)	1.16 ± 0.07^{a}	0.40 ± 0.20^{b}	1.00 ± 0.01^{a}	1.10 ± 0.04^{a}	-	0.97 ± 0.01^{a}	1.00 ± 0.06^{a}	1.09 ± 0.01^{a}	0.89 ± 0.01^{a}	1.04 ± 0.05^{a}
18:4n-3	-	0.24 ± 0.12^{a}	-	-	-	-	-	0.41 ± 0.01^{a}	-	-
20:3n-3	1.16 ± 0.06^{a}	1.02 ± 0.03^{a}	1.06 ± 0.15^{a}	1.19 ± 0.08^{a}	1.29 ± 0.11^{a}	1.26 ± 0.30^{a}	1.36 ± 0.29^{a}	0.90 ± 0.07^{a}	0.87 ± 0.01^{a}	0.87 ± 0.04^{a}
20:5n-3(EPA)	3.34 ± 0.10^{abc}	3.59 ± 0.04^{a}	2.73 ± 0.17^{cd}	3.30 ± 0.15^{abc}	3.16 ± 0.13^{abcd}	2.96 ± 0.22^{abcd}	3.10 ± 0.08^{abcd}	3.40 ± 0.07^{ab}	2.77 ± 0.04^{bcd}	2.65 ± 0.14^{d}
22:5n-3(DPA)	1.61 ± 0.02^{a}	1.22 ± 0.11^{a}	1.17 ± 0.08^{a}	1.54 ± 0.04^{a}	1.37 ± 0.07^{a}	1.67 ± 0.38^{a}	1.45 ± 0,03^{a}	1.16 ± 0.03^{a}	1.16 ± 0.00^{a}	1.34 ± 0.05^{a}
22:6n-3(DHA)	8.45 ± 0.20^{e}	19.26 ± 0.36^{b}	11.86 ± 0.27^{c}	7.13 ± 0.05^{f}	31.10 ± 0.30^{a}	11.63 ± 0.16^{c}	7.35 ± 0.22^{f}	19.85 ± 0.25^{b}	9.54 ± 0.14^{d}	6.32 ± 0.15^{f}
Σn-3PUFA	15.72 ± 0.20^{de}	25.73 ± 0.07^{b}	17.82 ± 0.10^{cd}	14.26 ± 0.27^{ef}	36.92 ± 0.50^{a}	18.49 ± 0.92^{c}	14.26 ± 0.58^{ef}	26.81 ± 0.15^{b}	15.23 ± 0.19^{e}	12.22 ± 0.33^{f}
ΣTYA	97.13 ± 0.03^{abc}	99.00 ± 0.55^{a}	97.92 ± 0.58^{abc}	98.42 ± 0.42^{ab}	97.79 ± 0.62^{abc}	98.81 ± 0.46^{a}	96.17 ± 0.24^{c}	98.35 ± 0.11^{ab}	98.71 ± 0.33^{a}	96.51 ± 0.25^{bc}
ΣTE	2.87 ± 0.03^{abc}	1.00 ± 0.55^{c}	2.08 ± 0.58^{abc}	1.58 ± 0.42^{bc}	2.21 ± 0.62^{abc}	1.19 ± 0.46^{c}	3.83 ± 0.24^{a}	1.65 ± 0.11^{bc}	1.29 ± 0.33^{c}	3.49 ± 0.25^{ab}
n-3/n-6	0.61 ± 0.02^{de}	1.19 ± 0.01^{b}	0.69 ± 0.02^{cd}	0.52 ± 0.01^{f}	1.85 ± 0.02^{a}	0.70 ± 0.04^{c}	0.54 ± 0.02^{ef}	1.21 ± 0.00^{b}	0.64 ± 0.01^{cd}	0.46 ± 0.01^{f}
DHA/EPA	2.54 ± 0.10^{ef}	5.36 ± 0.12^{b}	4.38 ± 0.29^{c}	2.17 ± 0.11^{f}	9.86 ± 032^{a}	3.97 ± 0.29cd	2.38 ± 0.012^{f}	5.83 ± 0.17^{b}	3.44 ± 0.00^{de}	2.40 ± 0.11^{f}
EPA/ARA	0.87 ± 0.05^{d}	1.03 ± 0.02^{bc}	0.90 ± 0.02^{cd}	0.80 ± 0.03d^{e}	1.16 ± 0.03^{b}	0.94 ± 0.05^{cd}	0.80 ± 0.02^{de}	1.39 ± 0.02^{a}	0.86 ± 0.02^{d}	0.70 ± 0.03^{e}

Table 1. Continue.

DHA/ARA	2.20 ± 0.14^{fg}	5.50 ± 0.07^{c}	3.91 ± 0.17^{d}	1.74 ± 0.04^{g}	11.44 ± 0.37^{a}	3.69 ± 0.12d^{e}	2.17 ± 0.23^{fg}	8.14 ± 0.15^{b}	2.94 ± 0.07^{ef}	1.66 ± 0.04^{g}
DHA/n-6DPA	2.55 ± 0.16^{a}	2.88 ± 0.03^{a}	2.65 ± 0.10^{a}	2.74 ± 0.07^{a}	2.79 ± 0.02^{a}	2.58 ± 0.14^{a}	2.72 ± 0.05^{a}	2.93 ± 0.02^{a}	2.61 ± 0.07^{a}	2.89 ± 0.07^{a}
n-6DPA/ARA	0.87 ± 0.11^{fg}	1.91 ± 0.02^{c}	1.48 ± 0.10d^{e}	0.64 ± 0.03^{g}	4.09 ± 0.17^{a}	1.54 ± 0.06^{cd}	0.71 ± 0.02^{g}	2.78 ± 0.04^{b}	1.13 ± 0.00^{ef}	0.58 ± 0.02^{g}

K indicates intensivelly cultured rotifiers, fed on a mixture of feed containing K-1+K-2; ZKA, ZKB and ZKC refers to the K group of rotifers which were harvested at the same times (6, 8 and 12 h) after treatments of three commercial enrichment products (ZA, ZB and ZC); and 0 refers to the K group of rotifers which were subjected to starvation for the same periods (6, 8 and 12 h, respectively) as the rotifers fed on three commercial enrichment products (ZA, ZB and ZC). Different letter in the same coloumn indicates significant differences ($p < 0.05$).

Single unsaturated fatty acid levels were lower in enriched rotifer groups than other experimental groups (13.46 ± 0.24% for ZA, 7.97 ± 0.73% ZB and 18.18 ± 0.16 % for ZC), whereas it was lowest in the rotifer group of K ($p < 0.05$) and was highest in ZOA starved group (Table 1).

The levels of total n–6 multiple unsaturated fatty acids in the groups of K, ZKA, ZKB and ZKC as well as in starved rotifer groups were higher than those of ZA, ZB and ZC rotifers ($p < 0.05$). The level of C16:4n–1 was measured as 1.53 ± 0.08% in Z0C group and was not detectable in the group of ZB. However, the level of C16:4n–1 in the group of Z0C was similar to that in the groups of K, Z0A and ZKC ($p > 0.05$). Highest level of linoleic acid was obtained from Z0A group and lowest from the group of ZB ($p < 0.05$). However, Z0A, Z0B and Z0C rotifers did not differ in their linoleic acid contents ($p > 0.05$). The level of linoleic acid in the enriched rotifer groups was lower than that in the remaining groups ($p < 0.05$) and similar levels of linoleic acid were observed between the groups of ZA and ZC rotifers ($p > 0.05$). Very low levels of γ-linolenic acid were only observed in the groups of ZA and ZC rotifers (Table 1).

The levels of C20:2n–6 fatty acids were highest in the group of ZKB rotifers (1.81 ± 0.03%), and was not detectable in ZB rotifers. Homo–g–linolenic (C20:3n-6) acids was low in commercially enriched rotifers, whereas this acid was high in the starved rotifer groups ($p < 0.05$).

ARA was highest in the Z0A rotifers (4.10 ± 0.07%) and lowest in the ZC rotifers (2.44 ± 0.03%). Except the group of ZA rotifers, all other experimental groups significantly differed in the ARA levels ($p < 0.05$). In addition, ARA levels were high in the starved rotifers (Table 1).

The level of n–6 DPA) was highest in the commercially enriched rotifers ($p < 0.05$). Highest value was obtained from ZB rotifers (11.12 ± 0.03%) ($p < 0.05$). Increasing the period of starvation led to remarkable decrease in the level of n–6DPA ($p < 0.05$) (Table 1).

Total n–3 multiple unsaturated fatty acids were high in enriched rotifer groups. Highest total n–3 PUFA was obtained from ZB rotifers (36.92 ± 0.50%) and lowest from Z0C rotifers (12.22 ± 0.33%) ($p < 0.05$). In the group of ZB rotifers, the total n–3 multiple unsaturated fatty acids mostly comprised of DHA.

Linolenic acid-αLNA was not detected in the ZB rotifers. The αLNA ranges from 0.40 ± 0.20% to 1.16 ± 0.07% in all the groups. The αLNA was detected as 0.24 ± 0.12% for ZA and 0.41 ± 0.01% for ZC. The differences in C20:3n–3 fatty acid levels between the experimental groups were insignificant ($p > 0.05$).

After and before enrichment, the levels of EPA were similar in all. EPA was 3.59 ± 0.04% for ZA (highest) and 2.65 ± 0.14% for Z0C (lowest). Highest level of n–3 dokosapentaenoic acid (C22:5n-3) was obtained from the group of K rotifers as 1.61 ± 0.02% and lowest from the group of ZC as 1.16 ± 0.30%, but the difference was not significant (Table 1).

Highest levels of DHA (C22:6n-3) was obtained from the group ZB and lowest from Z0C rotifers ($p < 0.05$). DHA level of ZB group (31.10 ± 0.30%) was higher than that of ZA (19.26 ± 0.36%) and ZC (19.85 ± 0.25%) ($p < 0.05$). The difference between ZA and ZC rotifers was insignificant ($p > 0.05$). The levels of HUFA ratios were higher in enriched rotifer groups (Table 1).

In this study, the lowest ratio of n–3/n–6 was obtained from the group of Z0C (0.46 ± 0.01%) and highest ZB rotifers (1.85 ± 0.02%). Enriched rotifers of ZA, ZB and ZC have higher levels of n–3/n–6 ($p < 0.05$), whereas the levels in ZA and ZB rotifer groups were similar ($p > 0.05$) (Table 1).

DHA/EPA ratio was highest in the ZB group (9.86 ± 0.32%) and lowest in the group of Z0A rotifers (2.17 ± 0.11%). The difference in DHA/EPA ratio between the group of ZB and the groups of K, ZKA, ZKB, ZKC, Z0A, Z0B and Z0C was significant ($p < 0.05$). The groups of ZA and ZC rotifers have similar DHA/EPA ratio ($p > 0.05$). EPA/ARA ratios were similar between the groups of ZA (1.03 ± 0.02%) and ZB (1.16 ± 0.03%) ($p > 0.05$), but lower than the ZC group (1.39 ± 0.02%) ($p < 0.05$). In contrary, it was low in the group of K rotifers (0.87 ± 0.05%) and Z0C rotifers (0.70 ± 0.03%), and the difference between these two

was significant ($p < 0.05$). DHA/ARA ratios increased in the enriched groups (highest in ZB 11.44 ± 0.37%) as compared to the Z0C rotifers (lowest 1.66 ± 0.04%) ($p < 0.05$). The difference between the groups of ZC and ZA was however significant ($p < 0.05$) (Table 1).

The ratio of DHA/n–6DPA was lowest in the K rotifers (2.55 ± 0.16%) and highest in the group of ZC rotifers (2.93 ± 0.02%), but the difference was insignificant ($p > 0.05$) (Table 1).

Ratio of n–6DPA/ARA increased by the enrichment products and was observed to be highest in the group of ZB rotifers (4.09 ± 0.17%) ($p < 0.05$). The values in the groups of ZA and ZC rotifers for the ratio of n–6DPA/ARA were 2.78 ± 0.04% and 1.91 ± 0.02%, respectively; and the difference was significant ($p < 0.05$). Lowest ratio of n–6DPA/ARA was obtained from the group of Z0C rotifers as 0.58 ± 0.02% (Table 1).

The differences between the groups of ZA, ZB and ZC rotifers in the ratios of n–3/n–6, DHA/EPA, DHA/ARA and n–6DPA and the difference between the groups of ZB and ZC rotifers in the ratio of EPA/ARA were significant ($p < 0.05$) (Table 1).

DISCUSSION

Total amounts of ΣMUFA obtained for all experimental groups including K group from this present study were lower from those values reported for non-enriched rotifers by Garcia et al. (2008a, b). The value of Σn–6PUFA for the K group rotifers was, however, higher than the values reported by Garcia et al. (2008a, b) and Westelmajer (2008).

The value of Σn–6PUFA obtained from ZA rotifers was higher than the values reported by Roo et al. (2009) and Westelmajer (2008), the value for ZB rotifers was similar to that reported by Cavalin and Weirich (2009) and Haché and Plante (2011), but higher than that of Garcia et al. (2008a, b).

The value of Σn–6PUFA obtained from the ZC rotifer group (22.09 ± 0.11%) was higher than that of Roo et al. (2009, 2010a,b) and Haché and Plante (2011). The reduced levels of Σn–6PUFA in the enriched groups compared to non-enriched groups was parallel to the same result by Garcia et al. (2008a), but enrichment had an increasing effect on Σn–6PUFA for the group of ZB in the work of Garcia et al. (2008b).

The values of LOA and ARA in K group rotifers in this study was higher than the values in the works of Garcia et al. (2008a), Westelmajer (2008) and Kotani et al. (2010) reported for the non-enriched rotifers. ARA values remained the same in the groups of non-enriched and starved rotifers (MacDonald, 2004; O'Brien–MacDonald et al., 2006). In this study, the reduced level of LOA in the groups of ZA, ZB and ZC rotifers was significant ($p < 0.05$).

LOA acid in the group of ZA rotifers was higher than the value reported by Roo et al. (2009). The value of ARA for the same group was similar to the value reported by Haché and Plante (2011). Both LOA and ARA in this study were lower than the values of Westelmajer (2008). Cavalin and Weirich (2009) reported low value of ARA and high value of LOA in comparison to our findings. The fatty acid content of n–6DPA obtained for ZA rotifers from our study was higher than those from Westelmajer (2008), Roo et al. (2009) and Cavalin and Weirich (2009).

LOA content (5.83 ± 0.21%) of ZB rotifers in this study was higher than the values of Garcia et al. (2008a b), Cavalin and Weirich (2009) and Haché and Plante (2011), and the content of ARA (2.73 ± 0.21%) was lower than the values of Cavalin and Weirich (2009) and Haché and Plante (2011). The values of n–6DPA in the group of ZB in this study was higher than the values of Garcia et al. (2008a, b) and Cavalin and Weirich (2009), and similar to those of Haché and Plante (2011).

LOA content of ZC rotifers in this study was higher than the values of Roo et al. (2009, 2010a, b), Naz (2008), Kotani et al. (2010) and Haché and Plante (2011), and the ARA content of ZC rotifers was lower than those of Roo et al. (2009, 2010a, b), Naz (2008), Kotani et al. (2010) and Haché and Plante (2011). The values of n–6DPA for ZC rotifers were higher than those of Roo et al. (2009) and Kotani et al. (2010).

The values of αLNA, except ZA rotifers, were similar in all groups ($p > 0.05$) and this was found to be agreed with the results of Garcia et al. (2008a), Naz (2008), Roo et al. (2009) and Kotani et al. (2010). The values of αLNA for ZB group with the values of Haché and Plante (2011) and Garcia et al. (2008a), and the values of αLNA for ZC with the values of Roo et al. (2010a,b) and Naz (2008) were similar.

EPA value of 3.34 ± 0.10% obtained from the group of K rotifers was higher than that of the same group reported by Garcia et al. (2008a, b), Naz (2008) and Westelmajer (2008).

EPA value of 3.59 ± 0.04 obtained from the ZA rotifers was similar to those reported by Roo et al. (2009) and Westelmajer (2008). EPA value of 3.16 ± 0.13 obtained from ZB rotifers was higher than those of Garcia et al. (2008a, b) and lower than those of Cavalin and Weirich (2009) and Haché and Plante (2011). In this study, EPA levels of ZB rotifers was lower than that of K rotifers, but these values were 3 and 3.5 fold higher that those of Garcia et al. (2008a, b). EPA was reduced in the ZC rotifers as compared to K rotifers. The EPA value of ZC rotifers (3.40 ± 0.07%) was similar to that of Naz (2008) and higher than that of Kotani et al. (2010), whereas it was lower than that of Roo et al. (2009, 2010a, b) and Haché and Plante (2011).

Similar values of n–3DPA were observed in all experimental groups. A value of 1.61 ± 0.02% for K rotifers was higher than those of Copeman (2001) and Westelmajer (2008). The value of n–3DPA in ZA rotifers was lower than those of Westelmajer (2008) and Cavalin

and Weirich (2009). A value of 1.37 ± 0.07% in the ZB rotifers was lower than those of Garcia et al. (2008a) and Haché and Plante (2011). The value of n–3DPA in the ZC rotifers was higher than that of Kotani et al. (2010) and lower than that of Haché and Plante (2011). The values of n–3DPA for the groups of ZA, ZB and ZC rotifers were similar to that of K rotifers ($p > 0.05$), but the values were 80% higher than that of Westelmajer (2008) for K group.

Higher level of DHA (8.45 ± 0.20%) for the non-enriched K group was always higher than the values reported by Copeman (2001), Garcia et al. (2008a, b), Naz (2008), and Westelmajer (2008). DHA content in ZA rotifer was similar to that of Roo et al. (2009) and was higher than that of Westelmajer (2008) and Cavalin and Weirich (2009). DHA level of ZB rotifers was higher than that of Garcia et al. (2008a, b) and Cavalin and Weirich (2009), and was lower than that of Haché and Plante (2011). DHA value of ZC rotifers (19.85 ± 0.25%) was similar to that of Naz (2008) and was higher than those of Kotani et al. (2010) and Roo et al. (2009, 2010a, b). DHA/EPA ratios in the enriched groups were similar to the ratios reported for several feed sources of larvae (~2:1 to ~1:1). This value was for marine flat fishes (DHA/EPA, ~2:1). For the diets of seabasses DHA/EPA, ratio was ~2:1 and 1:1; DHA/ARA ratio was ~10:1 or more (Sargent et al. 1999). DHA/EPA ratio was similar in the groups of ZA, ZB and ZC rotifers.

The ratio of n–3/n–6 in the group of K rotifers was lower than that of Garcia et al. (2008a, b), the ratios of DHA/EPA, EPA/ARA and *n*–6DPA/ARA for K rotifers were higher in this study. There were sporadic differences in these fatty acid profiles for non-enriched rotifers in the literature: Garcia et al. (2008b); Westelmajer (2008); Roo et al. (2009); Naz (2008); Haché and Plante (2011); Cavalin and Weirich (2009).

The crude fat contents of Red Pepper Paste, Algamac 3050 and Spresso were 13.5, 56.2 and 32%, respectively and their DHA/EPA ratios were 20, 15 and 9, respectively. In this study, Algamac 3050 had profound effect on the DHA contents of rotifers and on Σn–3PUFA, n–3/n–6 and DHA/EPA ratios. We personally recommend that a possible fortification of Algamac 3050 with ARA would lead to significant results on the enriched rotifers' fatty acid composition. The reason for the low ratios of n–3/n–6 in the rotifers enriched on Red Pepper Paste, Algamac 3050 and Spresso could be due to the high contents of LOA and n–6DPA fatty acids and the low contents of αLNA and EPA. Although, Red Pepper Paste has a high ratio of DHA/EPA, it did not lead to significant results on rotifers' fatty acid composition as compared to Spresso (similar result on DHA/EPA ratio) and Algamac 3050 (high result on DHA/EPA ratio).

Commercial enrichment products increased DHA level, but not EPA ratios of rotifers. ARA remained unchanged by Red Pepper Paste and reduced by other commercial products. There was a marked increased in the content of n–6DPA by all products, highest increase was obtained from the use of Algamac 3050. Enrichment did not affect the value of n–3DPA, but reduced the level of LOA. In all experimental groups the level of LNA was low.

Conclusion

In conclusion, the results reveal that the product of Algamac 3050 formulated as solid powder has positive effects on fatty acid profiles of rotifers as compared to other two commercial products, and therefore it increased nutritive value of rotifers.

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