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Research Article

The effect on fatty acid contents of Rotifer (*Brachionus plicatilis*) of Algamac 3050 and Olio ω -3 supplemented with or without L-Carnitine

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Abstract

This study determinated the enrichment effect of rotifers (*Brachionus plicatilis*) with Algamac 3050, Olio ω-3 and Selco parkle supplemented with L-carnitine on fatty acid composition. Rotifers were enriched with singly or in a combination of commercial products with or without L-carnitine; S. parkle, S. parkle+Algamac 3050, S. parkle+Olio ω-3, S. parkle+Algamac 3050+Olio ω-3, S. parkle+L-carnitine, S. parkle+Algamac 3050+L-carnitine, S. parkle+Olio ω-3+L-carnitine, S. parkle+Algamac 3050+Olio ω-3+L-carnitine. Considerable differences were found in the fatty acid composition of *B. plicatilis* fed with commercial enrichment diets. The highest EPA, DHA and PUFA were determined in rotifers enriched with S. parkle+Olio ω-3+L-carnitine, S. parkle+Algamac 3050+L-carnitine, S. parkle+Olio ω-3+Algamac 3050+L-carnitine, respectively at 6 hour. The enrichment duration was found to have significant effect on PUFA content of rotifers (p<0.05). This study clearly showed that L-carnitine can be used with commercial products to enrich the fatty acid contents of rotifers in marine fish hatcheries.

Keywords: *Brachionus plicatilis*, Fatty acid composition, L-carnitine, Algamac 3050, Olio ω-3

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Introduction

As the digestive systems of marine fish larvae are not fully developed during the first feeding period, rotifer is generally used as a starter food. Thus, rotifer is a key factor in the production of marine fish larvae (Lubzens *et al*., 2003). However, insufficient nutrient content of rotifers, especially meeting the requirements of essential fatty acids, reduces the survival rate of larvae (Holt, 2011). The fatty acid and population growth deficiencies of rotifers are corrected with use of natural sources, like phytoplanktons or commercial enrichment agents (Ferreira *et al*., 2008; Castillo *et al*., 2009; Mæhre *et al*., 2013; Eryalçın, 2018; Ozdogan and Savas, 2021; Ozdogan and Savas, 2022). Fatty acids are the major target nutrients in enrichment of rotifers, but some other key nutrients including essential amino acids and vitamins, like L- carnitine, are also enriched due to their roles in the growth of larvae (Harpaz, 2005). L-carnitine has attracted the attention of many researchers for decades because of its important functions in oxidation of long-chain fatty acids by mitochondria (Voet and Voet, 1995) and stimulation of protein-sparing action by dietary energy derived from lipids (Harpaz, 2005; Fathi and Farahzadi, 2014). Another important function of L-carnitine is to shuttle short-chain fatty acids from inside the mitochondria to the cytosol, thereby responsible for maintaining energy metabolism of the whole body level (Bremer, 1997).

Although there are many existing studies on effects of feeding live food supplemented with L-carnitine on the larval growth and survival rates of different fish species (Chen *et al*., 2011; Swagat *et al*., 2017), information on the effects of L-carnitine enrichment on live prey is lacking. Hence, the aim of this study was to determine the effect of different commercial enrichment products (Selco parkle, Olio ω-3 and Algamac 3050) with or without L-carnitine addition on the fatty acid contents of rotifer.

Materials and methods

Rotifer culture

Rotifer specimen (*Brachionus plicatilis*, L-strain 260-340 μm) were obtained from Mediterranean Fisheries Research, Production and Training Institute, Beymelek Center Unit, Antalya, Turkey. The trials were carried out at the Live Feed Laboratory of Egirdir Fisheries Faculty, Isparta University of Applied Sciences, Isparta, Turkey. Rotifers were cultured in 5-L containers under continuous illumination over 24 hours at the laboratory. Seawater used in the study was filtered through sand, cartridge, ultraviolet (280-315 nm) and biological filters. The salinity, temperature, dissolved oxygen and pH were adjusted to be 25‰, 25±1°C, 8.3 to 14.6 mg/L and 7.5±0.5, respectively.

Rotifer enrichment

The rotifers during mass cultures were fed with powdered microalgae of S. parkle (INVE Aquaculture Inc. USA). The commercial enrichment products used in the experiment were Olio ω-3 (BernAqua Inc. Belgium), Algamac

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3050 (Aquafauna Bio–Marine Inc. USA) and L-carnitine (Lonza Group, Switzerland). The nutritional composition of commercial products stated by the companies is given in Table 1.

Table 1: Nutritional composition of commercial products (Anonymous 2022a, b, c). EPA: Eicosapentaenoic acid, DHA; Docosahexaenoic acid.

Nutrient (%)	S. parkle	Olio ω-3	Algamac 3050
Moisture	58	34	2.1
Protein	2	-	17.6
Lipid	32	65	56.2
EPA	-	12	2.8
DHA	-	6	43.2

The control treatment in the experiment was Rotifers fed only with S. parkle whereas the other enrichment treatments were S. parkle+Algamac 3050 (Group I), S. parkle+Olio ω-3 (Group II), S. parkle+Algamac 3050+Olio ω-3 (Group III), S. parkle+L-carnitine (Group IV), S. parkle+Algamac 3050+L-carnitine (Group V), S. parkle+Olio ω-3+L-carnitine (Group VI), S. parkle+Algamac 3050+Olio ω-3+L-carnitine (Group VII). All treatments were tried in triplicated containers. Rotifers were enriched twice with the commercial products at doses recommended by the manufacturers, but L-carnitine was applied once at a concentration of 100 mg L-1 at the beginning of the enrichment process. After enrichment periods of 6 and 12 hours, rotifer samples were taken by filtering the enrichment medium. The samples were frozen and kept at -80°C until fatty acid analysis.

Fatty acid analysis

The lipid fractions of samples were extracted according to Bligh and Dyer (1959). The fatty acid percentages of lipids were analyzed by gas chromatography (Christie, 1989). The analyses were conducted in triplicate.

Statistical analyses

The statistical analyses of the experimental data were carried out using SPSS 21.0 software (SPSS, Inc., USA). The parameters for each feeding regimen were compared using one-way analysis of variance at a significance level of $p<0.05$ and the differences between the treatments were discriminated using Tukey's multiple comparison test. In addition, a two-way ANOVA analysis was carried out to test the effects of treatments, time (6 and 12 hours) and their interactions.

Results

According to the results of the experiment, significant differences ($p<0.05$) were determined among the treatments in terms of fatty acid contents of rotifers both after 6 and 12 hours of enrichment (Table 2). Long-chain fatty acids contents including EPA and DHA of the control group rotifers were significantly lower ($p<0.05$) than the other diet groups. EPA contents of rotifers enriched with Olio ω-3 were found to be higher than other experimental groups. DHA contents of rotifers enriched with Algamac 3050 were found to be higher than those in other experimental groups. A significant increasing effect of L-carnitine inclusion

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on the enrichment media of S. parkle+Olio ω-3, S. parkle+Algamac 3050 and, S. parkle+Olio ω-3+Algamac 3050 on EPA, DHA and total polyunsaturated fatty acids (PUFA) were determined, particularly at 6 hours after enrichment (Table 2).

Table 2: Effect on the fatty acid content (%) of rotifers supplemented with or without L-carnitine commercial products fed with 6 and 12 hours feeding time (mean±standard error).

Fatty Acids	Time (h)	Experimental groups*								Two-way Anova (*P* values)		
		Control	1	2	3	4	5	6	7	Treatment	Time	İnteraction
12:0	6	-	0,21± 0,04^{c}	0,59± 0,03^{a}	0,19± 0,03^{c}	0,43± 0,03^{b}	0,22± 0,01^{c}	0,23± 0,01^{c}	0,37± 0,02^{b}	0,001	0,001	0,001
	12	0,16± 0,01^{abc}	0,20± 0,04^{abc}	0,13± 0,02^{bcd}	0,25± 0,04^{a}	0,01± 0,03^{d}	0,11± 0,01^{cd}	-	0,22± 0,01^{ab}			
14:0	6	3,41 ± 0,01^{f}	4,89± 0,03^{c}	4,55± 0,07^{d}	8,56± 0,03^{a}	4,25± 0,01^{e}	3,22± 0,03^{g}	4,16± 0,03^{e}	7,81± 0,01^{b}	0,001	0,001	0,001
	12	4,85± 0,01^{f}	5,80± 0,03^{d}	5,13± 0,01^{e}	8,48± 0,03^{a}	6,3± 0,01^{c}	3,95± 0,01^{g}	5,15± 0,0^{e}	7,1± 0,01^{b}			
14:1	6	0,45± 0,01^{b}	0,15± 0,04^{d}	0,33± 0,02^{c}	0,07± 0,01^{d}	0,58± 0,01^{a}	0,34± 0,01^{c}	0,38± 0,01^{bc}	0,29± 0,05^{c}	0,001	0,001	0,001
	12	0,86± 0,04^{a}	0,58± 0,02^{b}	0,37± 0,05^{c}	0,35± 0,02^{c}	0,13± 0,01^{d}	0,79± 0,01^{a}	0,22± 0,01^{d}	0,65± 0,02^{b}			
16:0	6	15,56 ± 0,03^{a}	10,36 ± 0,02^{d}	10,45± 0,02^{d}	12,75± 0,01^{b}	12,85± 0,02^{b}	7,89± 0,05^{f}	9,89± 0,01^{e}	12,02± 0,05^{c}	0,001	0,110	0,001
	12	13,87± 0,02^{a}	8,41± 0,01^{g}	10,63± 0,04^{e}	13,64± 0,06^{b}	11,13± 0,01^{d}	10,04± 0,04^{f}	10,61± 0,06^{e}	13,33± 0,01^{c}			
16:1	6	9,01 ± 0,15^{c}	7,46± 0,01^{e}	8,50± 0,04^{d}	3,39± 0,03^{g}	12,39± 0,03^{a}	5,67± 0,02^{f}	9,24± 0,01^{b}	2,50± 0,07^{h}	0,001	0,239	0,001
	12	10,36± 0,04^{b}	6,24± 0,01^{e}	8,88± 0,06^{d}	3,37± 0,01^{g}	10,96± 0,02^{a}	5,54± 0,02^{f}	9,65± 0,05^{c}	3,24± 0,03^{g}			
18:0	6	5,67 ± 0,03^{c}	3,31± 0,01^{e}	8,96± 0,03^{a}	0,66± 0,02^{f}	5,62± 0,01^{c}	5,32± 0,01^{d}	7,82± 0,0^{b}	0,40± 0,03^{g}	0,001	0,001	0,001
	12	6,57± 0,02^{c}	4,23± 0,02^{e}	9,12± 0,01^{a}	0,71± 0,01^{g}	4,94± 0,03^{d}	4,09± 0,05^{f}	7,95± 0,06^{b}	0,5± 0,01^{h}			
18:1 n9	6	21,96 ± 0,01^{a}	7,96± 0,02^{c}	7,35± 0,04^{d}	5,49± 0,02^{f}	19,61± 0,01^{b}	6,32± 0,03^{e}	5,54± 0,02^{f}	4,66± 0,03^{g}	0,001	0,001	0,001
	12	22,10± 0,07^{a}	8,02± 0,04^{d}	6,97± 0,01^{e}	5,21± ,06^{f}	18,97± 0,04^{b}	7,89± 0,01^{d}	9,13± 0,01^{c}	4,99± 0,05^{g}			
18: 1 n7	6	5,73 ± 0,01^{b}	4,69± 0,02^{d}	5,05± 0,00^{c}	5,65± 0,05^{b}	2,56± 0,04^{e}	6,48± 0,02^{a}	5,71± 0,03^{b}	4,61± 0,01^{d}	0,001	0,001	0,001
	12	4,84± 0,03^{e}	5,25± 0,04^{d}	6,69± 0,03^{a}	6,52± 0,02^{b}	4,78± 0,01^{f}	4,79± 0,01^{f}	2,75± 0,02^{g}	5,84± 0,02^{c}			
18:2 n6	6	11,14 ± 0,01^{b}	2,63± 0,05^{g}	7,03± 0,01^{e}	8,37± ,02^{c}	12,85± 0,01^{a}	1,60± 0,07^{h}	5,55± 0,03^{f}	7,41± 0,01^{d}	0,001	0,001	0,001
	12	12,07± 0,01^{b}	1,09± 0,07^{f}	6,67± 0,03^{d}	7,81± 0,02^{c}	12,58± 0,04^{a}	1,05± 0,06^{f}	4,47± 0,04^{e}	7,92± 0,01^{c}			
18:3 n3	6	2,59 ± 0,05^{g}	5,86± 0,03^{c}	8,63± 0,02^{a}	5,34± 0,01^{d}	2,95± 0,04^{f}	4,42± 0,01^{e}	8,32± 0,01^{b}	4,35± 0,01^{e}	0,001	0,001	0,001
	12	1,27± 0,01^{g}	5,33± 0,01^{c}	6,38± 0,02^{a}	5,56± 0,06^{b}	3,99± 0,05^{e}	4,34± 0,02^{d}	3,28± 0,02^{f}	4,41± 0,01^{d}			

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Table 2 (continued):

Fatty Acids	Time (h)	Experimental groups*								Two-way Anova (*P* values)		
		Control	1	2	3	4	5	6	7	Treatment	Time	İnteraction
20:4 n6	6	0,85 ± 0,03^{e}	1,32± 0,01^{a}	1,03± 0,02^{d}	1,15± 0,04^{c}	0,75± 0,01^{f}	1,34± 0,01^{a}	1,18± 0,02^{bc}	1,26± 0,02^{ab}	0,001	0,145	0,001
	12	0,89± 0,01^{d}	1,01± 0,01^{c}	0,77± 0,01^{e}	1,4± 0,01^{a}	1,13± 0,01^{b}	1,22± 0,03^{b}	0,96± 0,05^{cd}	1,39± 0,03^{a}			
20:5 n3	6	4,75± 0,03^{h}	7,49± 0,03^{f}	18,75± 0,04^{b}	12,73± 0,06^{d}	5,75± 0,01^{g}	7,65± 0,03^{e}	22,29± 0,06^{a}	15,65± 0,03^{c}	0,001	0,001	0,001
	12	4,99± 0,01^{g}	7,15± 0,04^{f}	17,96±0 ,01^{b}	12,6± 0,02^{d}	4,8± 0,01^{h}	7,86± 0,05^{e}	20,22± 0,01^{a}	14,27± 0,02^{c}			
22:6 n3	6	7,86 ± 0,01^{e}	32,79± 0,04^{b}	7,45± 0,02^{f}	24,22± 0,04^{d}	7,96± 0,02^{e}	38,55± 0,03^{a}	7,87± 0,03^{e}	27,32± 0,01^{c}	0,001	0,001	0,001
	12	6,06± 0,04^{h}	35,61± 0,04^{b}	9,14± 0,06^{f}	22,8± 0,01^{d}	8,71± 0,01^{g}	36,76± 0,02^{a}	14,48± 0,01^{e}	25,11 ± ,2^{c}			
SFA	6	24,64 ± 0,07^{a}	18,77± 0,04^{e}	24,54± 0,05^{a}	22,16± 0,02^{c}	23,15± 0,08^{b}	16,64± 0,04^{f}	22,10± 0,08^{c}	20,59± 0,01^{d}	0,001	0,001	0,001
	12	25,44± 0,05^{a}	18,64± 0,03^{g}	25,01± 0,01^{b}	23,08± 0,01^{d}	22,38± 0,01^{e}	18,19± 0,01^{h}	23,71± 0,03^{c}	21,15± 0,02^{f}			
MUFA	6	37,15 ± 0,17^{a}	20,26± 0,04^{cd}	21,22± 0,02^{c}	14,60± 0,01^{e}	35,13± 0,05^{b}	18,80± 0,85^{d}	20,87± 0,04^{cd}	12,06± 0,04^{f}	0,001	0,001	0,001
	12	38,16± 0,13^{a}	20,09± 0,03^{e}	22,91± 0,06^{c}	15,45± 0,05^{g}	34,84± 0,01^{b}	19,01± 0,05^{f}	21,75± 0,05^{d}	14,72± 0,02^{h}			
PUFA	6	27,19 ± 0,05^{h}	50,09± 0,06^{d}	42,89± 0,04^{f}	51,80± 0,03^{c}	30,26± 0,01^{g}	53,55± 0,01^{b}	45,21± 0,07^{e}	55,98± 0,04^{a}	0,001	0,001	0,001
	12	25,27± 0,01^{g}	50,18± 0,01^{c}	40,92± 0,02^{e}	50,17± 0,07^{c}	31,21± 0,04^{f}	51,23± 0,09^{b}	43,41± 0,11^{d}	53,1± 0,05^{a}			

S. parkle (Control); S. parkle+Algamac 3050 (I); S. parkle+Olio ω-3 (II); S. parkle + Algamac 3050+Olio ω-3 (III); S. parkle+L-carnitine (IV); S. parkle+Algamac 3050+L-carnitine (V); S. parkle+Olio ω-3+L-carnitine (VI); S. parkle+Algamac 3050+Olio ω-3 + L-carnitine (VII), Different latter in the same columns indicates significant differences (p<0.05).

Comparing 6 and 12 hours of enrichment times of rotifers, it was found that enrichment time significantly affected PUFA content (p<0.05). Besides, when L-carnitine was added, saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) declined in rotifers which were enriched with commercial products but PUFA increased (Table 2). The highest PUFA level was determined in rotifer enriched with S. parkle+Olio ω-3+Algamac 3050+L-carnitine for 12 hours, and the differences among the groups were statistically significant (p<0.05). After 6 and 12 hours of enrichment, the PUFA levels of the rotifers enriched with L-carnitine added commercial products were significantly higher (p<0.05) than those without the addition of L-carnitine.

Two-way ANOVA analysis revealed that the treatment effects on all fatty acids and fatty acid groups were significant (p<0.05). When the effect of period or enrichment duration was considered, the levels of all fatty acids, except for 16:0, 16:1 and 20:4n-6, significantly changed between 6 and 12 hours. Significant P values were also detected for treatment×time interactions in all fatty acids and fatty acid groups, suggesting that the enrichment products differently changed the fatty acid profiles of rotifers with time.

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Discussion

In recent years, different commercial products have been developed to enhance PUFA contents of live foods and those products are widely used in marine finfish hatcheries. Rotifers that are used in feeding marine fish larvae must have high fatty acid content in terms of especially EPA and DHA to meet the larval requirements. Many researchers have studied and developed various enrichment products and enrichment procedures to increase the PUFA contents of rotifers (Saidi *et al*., 2018; Giménez Papiol and Estévez, 2019; Waqalevu *et al*., 2019; Matsui *et al*., 2022). The nutritional content of rotifers can be improved using microalgae, lipid emulsions and lipid-enriched microcapsules with short or long-term enrichment periods (Conceição *et al*., 2010). There are commercial products that are highly efficient in improving nutritional content of rotifers (Yang *et al*., 2009; Chen *et al*., 2010).

The results of the present study showed that rotifer enriched with Algamac 3050 significantly increased the DHA and PUFA level of rotifer compared with other treatments. Morever, this results are consistent with those reported by enrichment studies with Algamac products (Fu *et al*., 2021). On the other hand, EPA content of rotifers increased with the enrichment of Olio ω-3, which is similar to the results of the previous studies conducted by (Lundova *et al.*, 2018), who enriched artemia with Olio ω-3. The fatty acid profiles of the rotifers are a reflection of the content of EPA and DHA in the enrichments of Olio ω-3 and Algamac 3050 (Table 1) to rotifer in the current study.

The fatty acid content of rotifer groups enriched with commercial products supplemented with or without L-carntine were significantly different ($p<0.05$). It is stated that the fatty acid metabolism of fish fed with supplemented L-carnitine was effective (Harpaz, 2005). Rotifer enriched with L-carnitine showed an increase in terms of PUFA. EPA levels of rotifers in the present study are consistent with those reported by Cavalin and Weirich (2009), Carrier *et al*. (2011), Olivotto *et al*. (2011), and Boglino *et al*. (2014). DHA levels found in this study were similar or higher than those reported by Abu-Rezq *et al*. (2002), Haché and Plante (2011), Dantagnan *et al*. (2013), Ma and Qin (2014), and Hauville *et al*. (2016). Essential fatty acid content of rotifer enriched with commercial products supplemented with L-carntine was similar to studies mentioned above. Morever, EPA and DHA in fatty acid content of rotifer were comparable or higher than previous studies.

It is known that enrichment duration can affect the fatty acid contents of the live feed (Immanuel *et al*., 2007). However, there are studies reporting that enrichment time has no significant impact on fatty acid contents of live foods (Estévez and Giménez, 2017; Kotani *et al.*, 2017). In the present study, enrichment time significantly affected the essential fatty acids of rotifer. The highest EPA, DHA and PUFA were

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determined enriched with S. parkle+Olio ω-3+L-carnitine, S. parkle+Algamac 3050+L-carnitine, S. parkle+Olio ω-3+Algamac 3050+L-carnitine in rotifer respectively at 6 hours of enrichment. Increase of rotifers PUFA content was determinated enough enrichment duration of 6 hour's.

In conclusion, L-carnitine can be used in combination with high-fat commercial products while enriching the nutrient content of rotifers in aquaculture applications. This may positively contribute to the economic efficiency of marine finfish hatcheries. Future studies should be focused on the effects of rotifers enriched L-carnitine supplemented products on larval survival and growth performance.

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