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The Fatty Acid Composition of *Daphnia magna* Fed with Various Feeds

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Abstract: In this study, effects on fatty acid composition of *D. magna* produced in the different nutritional mediums (*S. acuminatus*, yeast, *S. acuminatus*+yeast, fish oil and yeast+fish oil) were investigated. Considerable differences were found in fatty acid composition of *D. magna* fed with algae, yeast and fish oil. *D. magna* fed with algae contains considerable amounts of PUFA (46.2%), though this algae is completely bare of EPA acids. *D. magna* fed with yeast was contained highest of MUFA (53.5%). In contrast to algae, yeast contains mainly 16:1 and 18:1 acids and was devoid of 18:3 or PUFA. *D. magna* fed with fish oil contained considerable amounts of PUFA especially, EPA and DHA acids. The rate of synthesis of these acids is rather low, although fish oil contains considerable amounts of these fatty acids.

Key words: *Daphnia magna*, algae, yeast, fish oil, fatty acid, composition

INTRODUCTION

Live food sources are essential in both marine and freshwater fish rearing especially during larval stages. The micro-algae and the zooplankton organisms are used as live food for fish larvae. The nutritional quality of live food is very important for the survival and growth of fish larvae. Food and feeding regime are important factors in zooplankton cultures such as rotifer, daphnia. Daphnia is used to feed of freshwater fish larvae. Therefore, along with the production of sufficient quantities of Daphnia, it is also necessary to ensure proper nutritional quality to satisfy the needs of the larvae. Foods can be different in mass culture of Daphnia such as micro algae, organic and inorganic manure, yeast supplemented with fish oil. Chemical composition of the microalgae using as food for Daphnia is probably more important than other factors. Because several biochemical constituents of animals cannot be synthesized de nova these organisms have a dietary requirement for such compounds which include essential fatty acid, essential amino acids and vitamins. The nutritional quality of microalgae will be poor if a specific fatty acid or an amino acid is absent. Some studies suggest that Polyunsaturated Fatty Acids (PUFAs) like eicopentaenoic acid (EPA, 20:5 ω3) and docosahexanaenoic acid (DHA, 22:6 ω3) may improve the quality of algae as food for cladocerans (Ahlgren *et al*., 1990; Lurling *et al*., 1997; Smyntek *et al*., 2008). To understand the importance of essential fatty acid for freshwater cladocerans, studies have been performed to investigate the effects of different diets, varying in fatty acid quality and quantity on body growth and fecundity as described in several papers (Demott and Muller-Navarra, 1997; Muller-Navarra, 2006; Von Elert and Wolfroom, 2001; Weers and Gulati, 1997). In this study, the fatty acid composition of *D. magna* is investegated that produced in different feeding regime using of microalgae and yeast. A supplementation approach is used to test the effects of fish oil in combination with algae and yeast to improve nutritional quality of the *D. magna* for aquaculture.

MATERIALS AND METHODS

The microalgae (*Scenedesmus acuminatus*) from green algae group was used in the study. The microalgae was cultured in 6-l bottles using Scenedesmus medium at 25°C. The cultures were continuously aerated by using a mini air pump and continual light provided by fluorescent lamps of 40 W. Algae in the log phase of growth was harvested for feeding and fatty analysis. Daphnia were cultured at 20°C and pH 7.0-7.5. The solutions were aerated by using a mini air pump and continual light. The initial stage of Daphnia cultivation was started in 500 mL flaks than the rest of production was continued consecutively in 2-l erlenmayer flaks and finaly in 6-l bottles. The experimental design for each media consist of 15 (3 replicate×5 food = alg, yeast, alg+yeast, alg+fish oil, yeast+fish oil) test bottles of 6-l capacity containing 5-l well water. Yeast was suspended in distilled water and 10 μg (about 1.5×10^4) was provided to *D. magna* each day. Fish oil, containing 15% EPA and 10% DHA were

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used for enrichment of *D. magna* as addition treatment. *D. magna*, previously fed on algae and yeast were enriched by adding 2 doses of 0.1 g L^{-1} to each culture for 24 h. Fish oil emulsions were prepared mixing the oil in 500 L of distilled water with a shaker for 15 min. Oil droplets of 1-3 μm diameters were formed through this process.

At the end of the experiment, the cultures were harvested and freeze-dried for fatty acid analysis. Total lipid was extracted from lyophilized algae cells and daphnia homogenate with chloroform/methanol (2:1, v/v) mixture according to the method of Bligh and Dyer (1959) and then saponified and methylated for fatty acid quantification according to the method modified by Garces and Mancher (1993).

The fatty acid was extracted with n-hexane and converted to methyl esters before injecting to gas chromatograph for analysis. Fatty Acid Methyl Esters (FAME) were analysed by Gas Chromatography (GC). Gas chromatography was performed on a QP 5050 Pelkin Elmer Aut System XLGC (GCndFID) using column CP SIL 88 FOR FAME (50 m×0.32 mm inner diameter; 0.25 μm film), FID detector at 250°C, automatically injector at 240°C and helium as the carrier gas. Column oven temperature conditions were as follows initial temperature of 60°C was held for 4 min and then increased 13°C min^{-1} to 175°C, then held constant for 27 min and finally increased 4°C min^{-1} to 250°C where it was held constant for 15 min. A quantity of 1 μL of the samples was injected on the column at a split rate of 1:70. Authentic fatty acid methyl esters were used for peak identification. The fatty acid methyl esters were reported as percent (peak area) of the total methyl fatty acid esters.

Statistical analyses were performed using SPSS 11.0 for windows software (SPSS Inc, Chicago, IL, USA). Differences in the fatty acid composition of Daphnia between treatments were tested using ANOVA. Data were analyzed by one-way Analysis of Variance (ANOVA) and multiple comparisons made Duncan's multiple range test. The significance level was at $p<0.05$.

RESULTS AND DISCUSSION

The relative fatty acid composition of the feeds used in *D. magna* culture was shown in Table 1. There were many similarities in the FA composition of feeds but also some important differences ($p<0.05$). The fatty acid, C16:0, C18:1 and C18:3 were clearly detected in the algae. The palmitic acid (C16:0) and linolenic acid (C18:3) percentages of algae were higher than those of yeast and fish oil. Yeast in contrast to algae, contained mainly C16:1, C18:1 and C18:2 acids and devoid of 18:3 or longer chain Polyunsaturated Fatty Acids (PUFA). In the green alga *S. acuminatus* no eicopentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were not detectable. Fish oil contained significant amounts of PUFA, particularly EPA and DHA.

Table 1: The total fatty acid compositions of different feeds

Fatty acids	Algae	Yeast	Fish oil
06:0	-	-	-
08:0	-	-	-
10:0	0.3±0.020	-	-
11:0	3.27±0.55	-	-
12:0	-	-	-
13:0	-	-	-
14:0	-	0.75±0.35	8.2±0.280
15:0	-	0.41±0.21	-
16:0	24.32±0.95	21.39±1.49	21.5±4.520
17:0	-	0.38±0.09	-
18:0	3.25±0.86	13.18±1.83	2.95±1.06
20:0	-	1.46±0.54	-
14:1	-	0.09±0.01	-
16:1	-	14.87±1.61	8.1±0.280
18:1	10.87±1.91	32.8±3.390	14.4±1.410
24:1n-9	-	-	-
18:2n-6t	-	1.02±0.10	-
18:2n-6c	17.05±1.41	4.20±0.91	1.55±0.07
20:4n-6	-	-	1.15±0.07
20:2n-6	-	-	0.55±0.53
18:3n-3	22.77±2.34	-	0.75±0.21
18:4n-3	-	-	3.00±0.28
20:5n-3	-	-	8.1±0.700
22:6n-3	-	-	11.05±0.35
ΣSFA	31.16±0.80[a]	63.25±3.87[a]	32.65±3.18[a]
ΣMUFA	10.87±1.91[c]	47.76±5.02[a]	22.5±1.690[b]
ΣPUFA	39.82±2.34[a]	5.23±0.80[b]	26.15±0.77[a]
Σw-6	-	5.23±0.80[a]	3.25±0.49[b]
Σw-3	22.77±2.34[a]	-	22.9±0.280[a]
ΣEPA	-	-	4.3±6.080
ΣDHA	-	-	6.15±8.69
w-3/w-6	-	-	7.04±1.01
EPA/DHA	-	-	0.69±0.48

[a]Mean±standard deviation. Values in the same row bearing different letters are significantly different ($p<0.05$)

The relative fatty acid composition of *D. magna* fed with different feeds was shown in Table 2. *D. magna* fatty acid concentrations and distributions were substantially different than them of feeds. Almost all fatty acid in algae present were determined compositions fatty acid of *D. magna*.

However, fatty acids of *D. magna* fed with algae were found no eicopentaenoic acid (EPA, 20:5 ω3) and traces docosahexaenoic acid (DHA, 22:6n-3). *D. magna* grown on yeast contain relatively large amount of MUFA (16:1 and 18:1 acids) while PUFA (C20 and C22) were no found. Cladocerans groups supplemented with fish oil contained significant amounts of EPA, one of the major long-chain PUFA. DHA is the second abundant long-chain PUFA in this groups but FA analysis of the total lipid fraction of *D. magna* showed that only traces of DHA were detectable. Moreover, DHA was not detected in *D. magna* fed with algae, yeast and algae+yeast.

Table 2: The total fatty acid compositions of daphnia fed with different feeds

Fatty acids	Algae	Yeast	Algae+Fish oil	Yeast+Fish oil	Algae+Yeast
06:0	-	-	0.07±0.000	-	-
08:0	-	0.58±0.02	0.07±0.000	-	0.56±0.08
10:0	0.13±0.000	0.3±0.000	0.06±0.000	-	0.4±0.000
11:0	-	0.29±0.00	-	-	0.3±0.000
12:0	-	-	0.07±0.000	-	-
13:0	-	-	-	-	-
14:0	1.87±0.370	2.15±0.51	8.45±0.180	6.37±1.15	1.50±0.02
15:0	0.53±0.000	-	0.74±0.070	0.85±0.09	-
16:0	19.34±0.020	14.92±0.11	21.13±0.14	19.63±0.87	15.01±0.80
17:0	0.46±0.070	-	0.59±0.040	0.27±0.00	-
18:0	3.1±1.1500	3.49±0.23	2.69±0.080	3.92±0.26	4.32±0.55
20:0	-	-	0.02±0.000	-	-
14:1	-	0.53±0.00	-	-	-
16:1	2.81±0.265	25.55±0.84	12.65±0.48	16.61±2.35	11.24±0.68
18:1	19.57±1.640	27.4±1.650	12.72±0.56	17.70±0.06	29.88±3.20
24:1n-9	1.5±0.5000	-	0.30±0.030	-	-
18:2n-6t	5.93±0.050	1.84±0.43	0.86±0.370	2.20±0.19	2.66±0.00
18.2n-6c	8.84±0.000	3.01±0.32	5.38±1.150	4.09±1.18	8.00±0.39
20:4n-6	-	0.88±0.75	0.60±0.000	0.92±0.10	0.55±0.17
20:2n-6	3.28±0.240	0.82±0.00	2.42±0.110	1.96±0.17	1.43±0.10
18:3n-3	26.68±1.360	7.50±0.12	7.08±0.250	10.64±2.84	15.50±0.99
18:4n-3	0.37±0.000	-	0.26±0.000	-	-
20:5n-3	-	-	8.43±0.690	6.71±0.08	-
22:6n-3	1.06±0.83	-	5.24±1.360	1.53±0.00	-
ΣSAFA	25.4 ± 0.800^{bc}	21.7 ± 0.100^{c}	33.9 ± 0.200^{b}	31.1 ± 1.700^{bc}	22.1 ± 1.400^{c}
ΣMUFA	23.9 ± 1.900^{c}	53.5 ± 0.800^{a}	25.7 ± 1.000^{c}	34.3 ± 2.300^{bc}	41.1 ± 2.500^{b}
ΣPUFA	46.2 ± 0.300^{a}	14.1 ± 0.500^{c}	30.3 ± 2.700^{b}	28.1 ± 4.200^{b}	28.2 ± 0.500^{b}
ΣW-6	18.1 ± 0.200^{a}	6.6 ± 0.600^{d}	9.3 ± 0.9000^{c}	9.2 ± 1.500^{cd}	12.7 ± 0.500^{b}
ΣW-3	28.1 ± 0.500^{a}	7.5 ± 0.100^{c}	21.1 ± 1.800^{b}	18.9 ± 2.800^{b}	15.5 ± 0.100^{b}
EPA	-	-	8.4 ± 0.7000^{a}	6.7 ± 0.100^{b}	-
DHA	1.1 ± 0.800^{b}	-	5.2 ± 1.4000^{a}	1.5 ± 0.100^{b}	-
w-3/w-6	1.55 ± 0.10^{b}	1.13 ± 0.10^{b}	2.26 ± 0.100^{a}	2.05 ± 0.10^{a}	1.22 ± 0.10^{b}
EPA/DHA	-	-	1.7 ± 0.3000^{b}	4.4 ± 0.100^{a}	

Mean±standard deviation. Values in the same row bearing different letters are significantly different ($p<0.05$)

The essential fatty acids are used by cladocerans for metabolic energy production have important structural and complex physiological roles (phospholipids) and regulate membrane fluidity. Their diets absence of these essential compounds such as linoleic and linolenic acids (C18:2 ω6 and C18:3 ω3, respectively) would certainly lead to deficiencies. The sdudies have show that Scenedesmus have non-detectable or trace amount of EPA and DHA while having substantial amounts of other PUFA such linoleic and linolenic acids (Ahigren *et al*., 1992; Isik *et al*., 1999; Boersma and Stelzer, 2000). In the study we were not detectable eicopentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6 n-3) in *S. acuminatus*. Linoleic and linolenic fatty acids are precursors for long-chained PUFAs such as EPA. Linoleic and linolenic acids are essential for almost all animals because they are not capable of synthesising linoleic acid (Weers and Gulati, 1997). Linolenic acid can be further metabolised into EPA and DHA. However, it is not know that whether cladocerans are able to convert linolenic acid into EPA and DHA. The study suggest that *D. magna* have capable of synthesizing linoleic acid (C18:2 ω6t and C18:2ω6c) whereas not including linoleic acid (C18:2 ω6t) in green algae *S. acuminatus*. Further, it was stated that enzymatic conversion activity (from 18:3 ω3 into 20:5 ω3) in the Daphnia fed on green algae (without EPA) might be sufficient to meet metabolic needs for EPA and to sustain rather fast growth. The study determined daphnids fed with algae are not able to convert C18:3 ω3 into longer, more unsaturated FA like EPA while have a little amount DHA (1.1%). It was stated that the SAFA 16:0 was typical of all the greens and unsatured 18:3 ω3 was generally more important in the green than the ω6 acids (Weers and Gulati, 1997). The results fatty acid of algae is showed similar with knowledge.

When daphnids with fed EPA free algae and yeast, they are not produce EPA but using dietary supplement fish oil daphnids are able to synthesize EPA under these feeding regime (Smyntek *et al*., 2008). Baker yeast in contrast to algae contained mainly 16:1 and 18:1 acids and devoid of longer chain saturated or polyunsaturated fatty acids in the study. Daphnids fed yeast contain relatively large amount of 16:1 and 18:1 acids and and devoid of C20 and C22 PUFA. It was stated that the yeast and *Scenedesmus* sp. are poor in ω3 fatty acids such as EPA and DHA and which are not an excellent source of essential fatty acids for freshwater cladocerans (Ahlgren *et al*., 1990; Lurling *et al*., 1997; Isik *et al*., 1999).

Adding droplets of fish oil which was rich in EPA and DHA, Daphnia fed with algae and yeast improved these fatty acids in the study. Weers and Gulati (1997) could improve the quality of the low long-chained PUFA alga Scenedesmus (undetectable EPA and DHA content) for *D. galeata* slightly by adding a PUFA-rich emulsion whereas an emulsion of more saturated and shorter fatty acids had no significant effect on food quality. As has been previously shown by Watanabe *et al.* (1983) the fatty acid composition of Daphnia is depend on their food. The result of fatty acid of *D. magna* show similar this knowledge.

CONCLUSION

In conclusion, when *D. magna* fed with *S. acuminatus* and yeast, it can be enriched with fish oil to improve nutritional quality of the *D. magna* for aquaculture. This information's related to effect different feeds on fatty acid composition of *D. magna* can be useful for aqua cultural potential for the hatchery rearing of larval stages of commercial fish species and aquarium fishes production.

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