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Comparison of the Nutritional Value of *Chlorella ellipsoidea* and *Nannochloris oculata* for Rotifers and *Artemia* Nauplii

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Microalgae are widely used for mass culture of the rotifer *Brachionus plicatilis* in aquaculture. Since the nutritional value of the rotifer is closely related to its food, the nutritional value of its food should be known in detail. *Chlorella ellipsoidea* and *Nannochloris oculata* are representative food organisms for rotifers that are easily cultured. Therefore, the nutritional values of these microalgae were examined for ultrasmall, small, and large rotifers and *Artemia* nauplii. *Chlorella ellipsoidea* contained seven times more total fatty acids than *N. oculata*. The three types of rotifer fed *N. oculata* contained more amino acids than those fed *C. ellipsoidea*. However, the total fatty acids of the rotifers fed each microalga species differed according to the type of rotifer. Newly hatched *Artemia* nauplii contained more protein and had a higher dry weight than those fed microalgae for 6 h. As with the rotifers, the *Artemia* nauplii fed *N. oculata* contained more protein and amino acids than those fed *C. ellipsoidea*, while the reverse was true for the total fatty acid content. Our results suggest that *N. oculata* is a good supply of protein, while *C. ellipsoidea* is a good source of lipids as food organisms for rotifers and *Artemia* nauplii in aquaculture.

Keywords: *Artemia* nauplius, *Chlorella*, *Nannochloris*, Rotifer

Introduction

In recent years, the importance of aquaculture for producing protein has increased worldwide. Since the success of aquaculture depends on the availability of seed, many studies have examined the rearing of larvae. The development of feeding regimes that allow the mass production of larvae is one of the barriers to the successful propagation of marine fish (Eda et al., 1990).

Larval nutrition has two distinct phases (Fraser et al., 1988): maternal nutrition affects fecundity and egg quality (Sargent et al., 1989; Watanabe et al., 1991; Furuita et al., 2002), and the larvae have their own nutritional requirements after depleting the yolk (Perez and Gatesoupe, 1988; Munilla-Moran et al., 1990; Furuita et al., 2003a, b). In both phases, the most important macronutrients are fatty acids, particularly n-3 HUFA (highly unsaturated fatty acid) (Watanabe et al., 1991; Watanabe and Vassallo-Agius, 2003; Mercier et al., 2004), and the quantity and quality of amino acids (Watanabe et al., 1984; Hayashi et al., 1985; Dendrinoos and Thorpe, 1987).

The rotifer *Brachionus plicatilis* and *Artemia* nauplii are the primary live foods for marine fish larvae and the nutritional values of these live foods are related to the microalgae they in turn eat (Whyte and Nagata, 1990).

In Korea, *Chlorella* (*i.e.*, *Nannochloropsis*) and *Nannochloris* are representative microalgal species used in the mass culture of rotifers in fish hatcheries because they are easy to culture and have high nutrient values (Cabrera and Hur, 2001). However, the nutritional differences between these microalgae species have not been fully examined. Therefore, this study compared the nutritional values of these microalgae to three types of rotifer and *Artemia* nauplii.

Materials and Methods

Chlorella ellipsoidea (KMCC C-20) and *Nannochloris oculata* (KMCC C-31) were obtained from the Korea Marine Microalgae Culture Center and

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cultured in f/2 media (Guillard and Ryther, 1962) in 20-L carboy bottles at 20 and 26°C, respectively, with aeration under continuous illumination (100 μmol/m^2/s) using cool-white fluorescent lamps. These microalgae were mass cultured in a 1-m^3 raceway with f/2 media. *Chlorella ellipsoidea* was cultured in an outdoor tank under natural light at temperatures around 15°C and *N. oculata* in an indoor tank under continuous illumination (60 μmol/m^2/s) using a cool-white fluorescent lamp at 26°C. Cells were harvested using a centrifuge and stored at -86°C.

Three types (ultrasmall, small, and large) of rotifer classified as *Brachionus plicatilis*, *Brachionus rotundiformis*, and *Brachionus* sp., respectively, which were used in the study of Cabrera et al. (1993), were cultured for several generations with *C. ellipsoidea* and *N. oculata* separately in round 100-L tanks. The rotifers were sieved through a 50-μm plankton net, washed, and stored at -86°C.

Artemia cysts (Utah strain; Golden West Artemia, Salt Lake City, UT, USA) were hatched in a 100-L conical tank at 25 to 27°C with continuous illumination (60 μmol/m^2/s). The hatched nauplii were sieved through a 100-μm plankton net and allowed to feed on *C. ellipsoidea* (4×10^6 cells/mL) or *N. oculata* (9×10^6 cells/mL) for 6 h at 21°C. Newly hatched nauplii and nauplii fed microalgae were collected using a 300-μm plankton net, washed, and stored at -86°C.

Frozen samples were freeze-dried in a vacuum freeze dryer (VFD-100; ULVAC, Methuen, MA, USA) and stored for later chemical analysis. To obtain the dry weight, 0.5-1.0 g of each sample was dried at 110°C until a constant weight was achieved. The total protein content of each non-freeze-dried sample was determined using the semimicro Kjedahl method (Joo et al., 1992).

To determine the amino acid composition, a 0.5-g sample was placed in a thick-walled glass hydrolysis tube; 2 mL of 6 N HCl and 50 μL of dimethyl sulfoxide were added; and the tube was kept in a sandbag at 110°C for 24 h. Then, the sample was lyophilized. The sample was then redissolved in loading buffer (0.2 N sodium citrate, pH 2.2) and filtered through a 0.22-μm filter (Millipore, Eugene, OR, USA). The amino acids in the sample were analyzed in 40 μL of this solution using an amino acid analyzer (LKB 4150-Alpha; Amersham Pharmacia, Buckinghamshire, England).

Total lipids were extracted from 1-g dry weight samples after freeze-drying according to Folch et al. (1957). The total lipid weight (±0.001 mg) was determined after extraction and evaporation. The dry samples were methylated using a mixed solution of chloroform and methanol (2:1, v/v). Then, hexane was added, and the samples were kept in a block heater at 100°C for 90 min, and evaporated to dryness under a stream of nitrogen gas. Eighty microliters of hexane were added to each sample, and the fatty acid composition was determined using a Perkin-Elmer 8700 gas chromatograph (Wellesley, MA, USA) equipped with a silica capillary column containing helium as the carrier gas, using heptadecanoic acid (17:0) as the internal standard (Slover, 1983).

Results

The fatty acid compositions of *C. ellipsoidea* and *N. oculata* were compared. The percentage of each fatty acid in the total saturated acids was similar in both microalgae. However, the total monounsaturated fatty acids were higher in *C. ellipsoidea*, while the total polyunsaturated fatty acids were higher in *N. oculata*. The total fatty acids in *C. ellipsoidea* were about seven times higher than in *N. oculata* (23.06 vs. 3.21 μg/100 μg dry weight) and the absolute amount of each fatty acid was higher in *C. ellipsoidea*. The major fatty acids detected in *C. ellipsoidea* were 16:0, 16:1, and 18:1, while those in *N. oculata* were 16:0, 18:3, and 20:3.

The fatty acid compositions of the three rotifers and *Artemia* nauplii allowed to feed on each microalga species were analyzed (Table 1).

The total lipid (μg/1,000 individuals) content of the rotifers ranged from 0.51 μg in the ultrasmall rotifers fed *C. ellipsoidea* to 17.05 μg in the large rotifers fed the same microalgae and depended on the type of rotifer and the microalga they were fed. The total lipids in the small rotifers were similar regardless of diet, while in the ultrasmall and large rotifers, the total lipids of the rotifers varied markedly with the diet. In all types, regardless of the diet, the major fatty acids were 16:1 and 18:1. The highest and lowest total polyunsaturated fatty acids as a percentage were detected in the ultrasmall rotifers fed *C. ellipsoidea* and the large rotifers fed *N. oculata*, respectively. Considering the absolute quantity of polyunsaturated fatty acids based on the total lipids, the highest amount (0.36 μg/100 μg dry weight) was detected in the large rotifers fed *C. ellipsoidea*.

In the large rotifer, the fatty acid distribution was similar regardless of the microalga species used as food. However, the absolute quantity of each fraction was higher in the rotifers fed *C. ellipsoidea*. In the small rotifer, the total saturated fatty acids were higher in those fed *C. ellipsoidea* versus *N. oculata*,

Table 1. Fatty acids composition (%) of three types of rotifer *Brachionus* and *Artemia* nauplii, which were cultured or enriched with *Chlorella ellipsoidea* or *Nannochloris oculata*

Fatty acids	Large type		Small type		Ultra-small type		*Artemia* nauplii		
	Ce	*No*	*Ce*	*No*	*Ce*	*No*	*Ce*	*No*	NH
14:0	7.40	4.96	5.41	4.40	4.98	1.34	-	-	-
16:0	35.18	28.67	31.74	-	-	35.18	2.55	2.43	2.45
18:0	5.98	5.89	5.60	2.88	-	6.42	-	-	-
20:0		-	0.37	-	-	1.16	-	-	-
22:0	-	-	1.58	-	-	-	2.68	5.04	1.76
Total saturated	- 49.62	40.63	44.71	7.28	4.98	45.85	5.23	7.46	4.21
14:1n-5	-	-	0.56	9.10	8.91	1.47	2.46	2.73	2.45
16:1n-7	25.37	6.38	19.07	55.76	39.76	24.14	36.37	35.09	35.01
18:1n-7+n-9	11.44	15.90	10.27	4.82	13.65	11.33	22.90	24.62	16.94
20:1n-7+n-9	2.61	1.46	3.48	-	-	5.19	-	-	-
Total monounsaturated	39.42	52.11	33.37	69.68	62.32	42.12	61.73	62.44	54.41
18:2n-6	5.67	4.06	4.50	6.67	32.71	5.46	33.04	30.10	27.25
18:3n-3+n-6	-	0.76	-	13.74	-	-	-	-	11.87
20:2	-	0.32	-	2.63	-	-	-	-	-
20:3n-6	-	-	8.49	-	-	-	-	-	-
20:4n-3+n-6	4.99	1.70	4.39	-	-	4.21	-	-	2.26
20:3n-6	-	0.42	2.63	-	-	-	-	-	-
20:5n-3	-	-	1.91	-	-	2.36	-	-	-
Total polyunsaturated	10.66	7.26	21.92	23.04	32.71	12.03	33.04	30.10	41.38
Total fatty acids (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total fatty acids (µg/100 µg dry weight)	3.41	0.56	1.13	1.31	0.39	2.20	3.28	1.56	3.34

Ce, fed *C. ellipsoidea*; *No*, fed *N. oculata;* NH, newly hatched nauplii; -, not detected.

while the total monosaturated fatty acids were lower in those fed *C. ellipsoidea*. The composition of total polyunsaturated fatty acids was similar in those fed either microalga. In the ultrasmall rotifer, the percentages of total monounsaturated and polyunsaturated fatty acids in the rotifers fed *C. ellipsoidea* were higher than in those fed *N. oculata*, although the absolute quantities of total lipids and fatty acids were lower when *C. ellipsoidea* was used as food.

The total fatty acid (µg/100 µg dry weight) contents of newly hatched nauplii and those fed *C. ellipsoidea* were similar, while they were lower in the nauplii fed *N. oculata*. Newly hatched nauplii had a higher total polyunsaturated fatty acid content than those fed microalgae and 18:3n3+n6 fatty acid was mainly detected. The distributions of total fatty acids as percentages were similar in the nauplii fed microalgae, but the absolute quantities of monounsaturated and polyunsaturated fatty acids were higher in nauplii fed *C. ellipsoidea* versus *N. oculata*.

The amino acid compositions of the three rotifers and *Artemia* nauplii fed each microalga species were analyzed (Table 2). The lowest total amino acid composition was 6.12 µg/1,000 individuals in the ultrasmall rotifer fed *C. ellipsoidea*, and the highest was 118.75 µg/1,000 individuals in the large rotifer fed *N. oculata*. In each rotifer species, the amino acid composition of the rotifer fed *N. oculata* was always higher than in those fed *C. ellipsoidea*. The large and small rotifers fed *N. oculata* had about twice the amino acid content of rotifers fed *C. ellipsoidea*. In contrast, in the ultrasmall rotifer, the total amino acid contents were similar on both microalgae diets. Notably, there was more arginine in the small rotifer than in the large rotifer.

In newly hatched *Artemia* nauplii and nauplii fed microalgae, the moisture content varied between 93 and 95%. The individual dry weight was higher in newly hatched nauplii than that in those fed microalgae. The protein content was similar in newly hatched nauplii (17.7%) and those fed *C. ellipsoidea* (17.46%) for 6 h, but was higher in the nauplii fed *N. oculata* (21.19%).

Regarding the amino acid composition, the total amino acids were highest (16.88 µg/1,000 individuals) in newly hatched nauplii and lowest (12.67 µg/1,000 individuals) in nauplii fed *C. ellipsoidea*; the nauplii fed *N. oculata* were similar to the newly hatched nauplii. Notably, the newly hatched nauplii had a higher arginine content (2.34 µg/1,000 individuals) than the nauplii fed microalgae.

Table 2. Amino acids composition (μg dry weight /1,000 individuals) of three types of rotifer *Brachionus* and *Artemia* nauplii, which were cultured or enriched with *Chlorella eliipsoidea* or *Nannochloris oculata*

Amino acids	Large type		Small type		Ultra-small type		*Artemia* nauplii		
	Ce	*No*	*Ce*	*No*	*Ce*	*No*	*Ce*	*No*	NH
Aspartic acid	6.60	11.35	3.03	9.08	0.76	0.60	1.25	1.37	1.38
Threonine	3.35	6.90	1.33	3.88	0.38	0.31	0.77	0.92	0.93
Serine	3.85	7.05	1.60	4.45	0.41	0.36	0.74	0.94	1.01
Glutamic acid	9.10	15.55	3.37	9.45	0.80	0.71	1.71	2.18	2.11
Proline	3.65	8.60	2.58	4.05	0.30	0.77	0.70	1.10	1.09
Glycine	3.10	4.45	1.29	3.30	0.39	0.34	0.68	0.81	0.75
Alanine	2.35	4.45	1.19	2.52	0.26	0.22	0.58	0.81	0.70
Cystine	2.15	3.65	0.99	2.52	0.26	0.09	0.02	0.43	0.41
Valine	3.65	8.45	1.84	4.05	0.45	0.54	0.83	1.13	0.92
Methionine	0.30	0.05	0.00	0.78	0.02	0.02	0.01	0.04	0.01
Isoleusion	3.15	6.20	1.46	3.67	0.26	0.28	0.68	0.90	0.77
Leucine	6.00	11.10	2.89	6.97	0.51	0.49	1.11	1.40	1.34
Tyrocine	-	-	-	-	-	-	0.14	-	-
Phenilalanine	3.55	7.65	1.67	3.88	0.29	0.31	0.83	0.99	0.88
Histidine	4.45	6.40	2.24	4.83	0.38	0.29	0.67	0.88	1.00
Lysine	4.90	8.55	2.24	5.20	0.34	0.31	0.94	1.21	1.23
Arginine	4.20	7.35	4.39	8.70	0.33	0.66	1.00	1.25	2.34
Total	64.35	118.75	32.10	77.32	6.12	6.29	12.67	16.35	16.88
EAA	33.55	62.65	18.05	41.96	2.95	3.21	6.85	8.72	9.42
NEAA	30.80	56.10	14.04	35.36	3.17	3.08	5.82	7.64	7.45
EAA/NEAA	1.09	1.12	1.29	1.09	0.93	1.04	1.18	1.14	1.26

Ce, fed *C. ellipsoidea*; *No*, fed *N. oculata*; NH, newly hatched nauplii; EAA, essential amino acids; NEAA, non- essential amino acids.

Discussion

The general proximate chemical composition of an organism is closely related to that of its food (Ben-Amotz et al., 1987; Frolov et al., 1991; Cho et al., 2001). Microalgae such as *Chlorella* and *Nannochloris* are widely used as food organisms for rotifers due to their high nutritional value and ease of mass culture.

The chemical composition and nutritional quality of microalgae vary inter- (Volkman et al., 1989) and intraspecifically according to the culture medium and growth stage (Borowitzka, 1988; Phatarpekar et al., 2000). In this study, the total lipid content was higher in *C. ellipsoidea* than in *N. oculata*, and it was similar to the value reported by Hur and Kim (1988) for the same species. Compared to *C. ellipsoidea*, Hur et al. (1989) also detected high levels of fatty acid 18:3 in *N. oculata*. Although the percentage of total polyunsaturated fatty acids (PUFA) to the total fatty acids was higher in *N. oculata* than in *C. ellipsoidea*, the total lipid content of the former was approximately seven times lower than that of the later. This relationship (high PUFA percentage and low lipid content) may affect the food value of microalgae (Yoon et al., 1989).

Nannochloris oculata had a high level of 18:3 fatty acid, which was not detected in *C. ellipsoidea*. Nevertheless, Fukusho et al. (1985) found that this fatty acid was not important in flounder larvae. The growth and survival of larval flounder fed on rotifers cultured with *N. oculata* were also inferior to those fed rotifers cultured with *C. ellipsoidea* (Cabrera et al., 1993). This also indicates that 18:3 fatty acid is not essential for flounder larvae.

Regarding the amino acid contents of the three rotifer species cultured with microalgae, the total amino acids were the highest in the large rotifer and lowest in the ultrasmall rotifer. The content of the small rotifer was only 50% (fed *C. ellipsoidea*) to 65% (fed *N. oculata*) that of the large rotifer. Notably, the small rotifer contained more arginine than the large rotifer. In addition, regardless of type, the rotifers cultured with *N. oculata* had a higher content than those cultured with *C. ellipsoidea*.

Cabrera and Hur (2001) found that the total protein and lipid of rotifers varied with rotifer species, which differed in size, and that different rotifers needed different microalgae species for their growth (Cabrera et al., 2005). This could be explained by genetic differences in the strains (Fu et al., 1991), each of which is considered a different species (Rumengan et al., 1991). When we consider the percentage of total polyunsaturated fatty acids detected, the small rotifer appeared to have the highest nutritional value.

However, the absolute lipid content and the interaction between total lipids and total polyunsaturated fatty acids should also be considered.

Comparing the nutritional composition of newly hatched *Artemia* nauplii with those fed microalgae for 6 h, the nutritional value of the nauplii decreased with time since hatching.

The dry weight of nauplii fed *N. oculata* was 80% that of newly hatched nauplii, while the protein content of the former was 1.2 times higher than that of the latter. In addition, the total amino acid content of the nauplii fed *N. oculata* was 1.29 times higher than that of nauplii fed *C. ellipsoidea*. The arginine content of the nauplii fed microalgae decreased markedly compared to newly hatched nauplii, indicating that this amino acid is a major nutrient for newly hatched nauplii.

Conversely, the nauplii fed *N. oculata* had fewer total fatty acids than those fed *C. ellipsoidea*. The total fatty acid content of the former was only 48% that of the latter. The polyunsaturated fatty acid composition (%) in the nauplii fed microalgae was only 30-33% of the total fatty acids.

Comparing the fatty acids compositions (%) of newly hatched nauplii and those fed microalgae, while saturated and monounsaturated fatty acids were higher in the nauplii fed microalgae, polyunsaturated fatty acids were higher in newly hatched nauplii. Therefore, polyunsaturated fatty acids are primarily consumed by nauplii compared to other fatty acids. The results for *Artemia* nauplii paralleled those of the rotifer experiment. The nutrient composition of *Artemia* nauplii was also affected by the diet.

In conclusion, we showed that *C. ellipsoidea* and *N. oculata* have different nutritional values. We recommend that *N. oculata* be used to supply protein and *C. ellipsoidea* be used to supply lipids as the food organisms in aquaculture.

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