Effects of commercial enrichment diets on the nutritional value of the rotifer (Brachionus plicatilis)

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ABSTRACT

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The rotifer, Brachionus plicatilis, pre-fed on baker's yeast, was enriched for 6 h with three commercial products from Artemia Systems S.A., namely Protein Selco (microcapsules), Dry Selco (microparticles) and Super Selco (an emulsion containing high levels of n-3 HUFA). The biochemical composition (protein, carbohydrate, total lipid, lipid classes and fatty acids) and dry weight of the rotifers before and after the enrichment experiments were studied. Two of the enrichers, Dry Selco and Super Selco, are rich in lipid but poor in protein and carbohydrate. Protein Selco contains lipid as well as protein and carbohydrate. The biochemical composition and the dry weight of the rotifers were changed after 6 h of enrichment. The percentage of protein and lipid increased with all of the three enrichers and the percentage of carbohydrate decreased slightly. Rotifer dry weight increased in rotifers enriched on Protein Selco. Phospholipids, sterol esters+ waxes, triacylglycerols and free fatty acids increased after the enrichment with the three enrichers while sterols slightly decreased when the rotifers were fed on Protein Selco. The fatty acid content of the rotifers increased after the enrichment with all of the enrichers and this increase was particularly apparent in the case of n-3 HUFAs (20:5n-3 and 22:6n-3).

INTRODUCTION

The rotifer, Brachionus plicatilis, has been widely used as a food during the first development stages of fishes and crustaceans since the first experiments on its culture were published by Ito (1960). Since then, the nutritional quality of the rotifer has been studied by many authors (Watanabe et al., 1978, 1983; Ben-Amotz et al., 1987; Dendrinos and Thorpe, 1987; Fernandez-Reiriz et al., 1987; Lubzens et al., 1987). However, fish larvae fed on rotifers often exhibit slow growth rates and high mortalities which has been attributed to PUFA deficiencies (Owen et al., 1975; Cowey, 1976; Howell, 1979; Scott and Middleton, 1979; Watanabe et al., 1983; Ben-Amotz et al., 1987; Dendrinos and Thorpe, 1987; James et al., 1987; Kissil and Koven, 1990). The chemical composition of the rotifer has been shown to be altered by diet (Watanabe et al., 1983; Frolov et al., 1991). Furthermore, a relationship between the fatty acid content in the diet and the rotifer has also been reported (Watanabe et al., 1978, 1983; Scott and Middleton, 1979; Lubzens et al., 1985, 1987; Estevez and Planas, 1988; Stottrup, 1989; Frolov et al., 1991).

Mass culture of rotifers is usually carried out on baker's yeast, Saccharomyces cerevisiae. However, rotifers fed exclusively on baker's yeast are often nutritionally deficient when used as food for fish larvae. Many attempts have been made to improve the nutritional quality of the rotifers in the last few years, and two enrichment methods have been described: the "indirect method" which attempts to improve the biochemical composition of the yeast by changing its culture medium (Imada et al., 1979) and the "direct method" which supplies the rotifers with baker's yeast plus some specific nutritional compounds, mainly emulsions of marine oils (Watanabe et al., 1983). During the last few years, various diets such as microalgae, lipid emulsions, microparticulate or microcapsules containing lipids or lipids plus proteins and carbohydrates have been used to enrich rotifers previously fed on baker's yeast (Gatesoupe and Luquet, 1981; Walford and Lam, 1987; Leger et al., 1989; Nichols et al., 1989; Rodriguez-Rainuzzo et al., 1989; Kissil and Koven, 1990; Linares et al., 1990).

This paper reports the chemical composition (proteins, carbohydrates, total lipids, lipid classes and fatty acids) of the rotifer, Brachionus plicatilis, after 3 and 6 h of enrichment with three commercial products: Protein Selco, Dry Selco and Super Selco from Artemia Systems. The composition of the enriched and non-enriched rotifers is

compared in order to establish the suitability of these live organisms as potential food for fish larvae, particularly turbot larvae.

MATERIAL AND METHODS

Culture of rotifers

The rotifers, Brachionus plicatilis, previously fed on baker's yeast (1 g/10^6 rotifers day^{-1}) were enriched for 3 and 6 h with three commercial products from Artemia Systems: Protein Selco (microcapsules), Dry Selco (microparticles) and Super Selco (an emulsion containing high levels of n-3 HUFA) .

The enrichment experiments were carried out in 171 tanks with a density of 600 rotifers per ml. The enrichment was made by adding 2 doses of 0.189 g/l to each culture. The first dose was added to the cultures at the beginning of the experiment and the second after 3 h. The enrichments were carried out at a temperature of 20ºC. Two replicates were made for each experiment.

The cultures were sampled at the beginning of the experiments and after 3 and 6 h of enrichment for various biochemical analyses. Volumes of water with a quantity of 1 x 10^6 rotifers were filtered out. The samples were then gently washed with distilled water to eliminate both salt and possible residues of enricher. They were frozen in liquid nitrogen and later freeze-dried and frozen until corresponding biochemical analyses were performed.

Analytical methods

Protein was assayed as described by Lowry et al. (1951) after hydrolysis with 0.5 N NaOH for 24 h at 30°C. Total carbohydrates were quantified as glucose by the phenol-sulfuric acid method (Strickland and Parsons, 1968). Lipids were extracted by the method of Bligh and Dyer (1959) as modified by Fernandez-Reiriz et al. (1989). Total lipids were determined gravimetrically. Lipid classes were studied by thin layer chromatography (TLC)-densitometry. Silicagel 60W plates (Merck 16486) with a size of 20 x 20 cm and a layer thickness of 0.25 mm were used. The chromatographic stain

was made by following the technique described by Freeman and West (1966). Samples were applied on the plate with the help of an automatic TLC sampler (Camag 27220). The plates were stained with a 10% $CuSO_4$ solution in 0.85% H_3PO_4 by heating to 180°C (Bitman and Wood, 1982). For the quantitative analysis, cholesterol palmitate, cholesterol, palmitic acid and tripalmitin (Sigma) were used as standards for sterol esters + waxes, sterols, free fatty acids and triacylglycerides, respectively. For phospholipids a standard obtained from rotifers was used. The plates were scanned with a Shimadzu CS9000-densitometer, using a beam monochromatic 370 nm of 0.4 x 0.4 mm working in the zig-zag mode, reading the whole spot, and with automatic autozero for base line correction.

Fatty acids from total lipid were transesterified to methyl esters with methanolic hydrogen choride as described by Christie (1982) and subsequently analyzed by gas chromatography as previously described by Fernandez-Reiriz et al. (1989), with the only exception that a PTV cold injector (Perkin-Elmer), operating in the solvent elimination mode as described Herraiz et al. (1987) was used. Nonadecanoic acid was used as an internal standard and a response factor was calculated for each fatty acid in order to perform quantitative analysis.

The caloric content of rotifers was calculated using the conversion factors given by Brett and Groves (1979).

A Fisher PLSD test was applied for statistical analyses at a confidence level of 95% ($P<0.05$).

RESULTS

Dry weight and proximate composition (proteins, carbohydrates and total lipids)

The dry weight (as ng/ind.) and proximate composition (as % dry weight) of B. plicatilis and the enricher are shown in Table 1. Only one of the three enrichers, Protein Selco, had appreciable quantities of protein and carbohydrate (around 30% each) with about 30% of lipid. Dry Selco and particularly Super Selco contained mainly lipid (56.1 and 94.2%, respectively). With respect to the rotifers, the percentages of protein and

lipid increased significantly ($P< 0.05$) after 6 h of enrichment with all of the three products. The percentages of carbohydrate decreased significantly ($P< 0.05$) after the enrichment.

The dry weight of the rotifers fed on Protein Selco increased significantly ($P<0.05$) after 6 h (453.5 rig/inn.). This increase represents 22.7% of the value found in non-enriched rotifers. The dry weight decreased significantly ($P<0.05$) 14.3% (316.5 ng/ind.) if the enrichment was made on Super Selco. No significant difference ($P>0.05$) was found in the dry weight of the rotifers fed on Dry Selco. These differences in dry weight will affect the absolute values of the biochemical compounds. In this sense, the content of protein and carbohydrate of Protein Selco-enriched rotifers increased significantly ($P< 0.05$) 29.7% and 14.3% with regard to the values found in non-enriched rotifers but decreased significantly ($P<0.05$) if the rotifers were enriched with Super Selco (6.1 and 33.0%, respectively). In Dry Selco-enriched rotifers, the protein content increased significantly ($P<0.05$) 7.7% and carbohydrate decreased significantly ($P < 0.05$) 10.9%. The lipid content of the rotifers increased significantly ($P< 0.05$) in the three experiments (116.5, 74.9 and 62.396, for Protein, Dry and Super Selco, respectively).

Lipid classes

Table 2 shows the composition (mg/g dry weight) of lipid classes of the three enrichers and the rotifers before and after the enrichment experiments. Protein Selco contained alkyl glyceryl ethers, triacylglycerols, free fatty acids and phospholipids. Dry Selco contained alkyl glyceryl ethers, phospholipids and free fatty acids and Super Selco alkyl glyceryl ethers and phospholipids. Alkyl glyceryl ethers were the major lipid class in the three enrichers although these lipids were not found in the rotifers even after 6 h of enrichment. Phospholipids were the major lipid class in the non-enriched rotifers, followed by sterol esters + waxes, triacylglycerols (TAG), free fatty acids (FFA) and sterols. Phospholipids, sterol esters + waxes, TAG and FFA increased signiticantly ($P<0.05$) after the enrichment in the three experiments while sterols decreased significantly ($P< 0.05$) when the rotifers were fed on Protein Selco.

Fatty acid composition

Table 3 shows the fatty acid composition (mg fatty acid/g dry weight) of the three enrichers and B. plicatilis without enrichment and after enrichment for 3 and 6 h. Super Selco had the highest quantity of unsaturated fatty acids. Dry Selco had the highest content of saturated fatty acids. Protein Selco had the lowest fatty acid content. In the non-enriched rotifers, the major fatty Acids were 18:1(n-9), 16:1(n-7), 18:2(n-6), 16:0, 18:0, 20:5(n-3), 22:6(n-3), 20:1(n-9), 18:1(n-7), 18:1(n-11), 20:3(n-6) 22:5(n-3), 20:2(n-6) and 20:4 (n-6). After the enrichment, the same fatty acids were found but the relative percentages were different depending on the enricher. In Protein Selco-enriched rotifers, the values of each fatty acid increased significantly ($P< 0.05$) after 3 h and did not change after 6 h with the exception of 20:5(n-3) which showed a significant increase ($P< 0.05$). In Dry Selco and Super Selco-enriched rotifers, the fatty acids increased significantly ($P< 0.05$) during the enrichment period with the highest values detected after 6 h.

Caloric content

Table 4 shows the caloric content of the rotifers before and after the enrichment. The highest total caloric content was observed after 6 h, with values of 2.00, 1.62 and 1.42 Cal/ 10^3 rotifers, for Protein, Dry and Super Selco, respectively.

DISCUSSION

At the end of the experiments the dry weight of rotifers enriched with Protein Selco increased significantly ($P< 0.05$). In the case of Super Selco-enriched rotifers, there was a significant decrease ($P< 0.05$) in dry weight after the enrichment. No significant difference (P>O.O5) was found in the dry weight of Dry Selco-enriched rotifers. These results suggest that Dry and Super Selco could be given to the rotifers together with baker's yeast to avoid a weight loss. Protein Selco-enriched rotifers showed a significant increase ($P< 0.05$) in the absolute values of protein and carbohydrate. In Dry Selco-enriched rotifers, proteins increased significantly ($P< 0.05$) and carbohydrates decreased significantly ($P< 0.05$). Super Selco-enriched rotifers depleted their own reserves probably due to the lack of protein and carbohydrate in the enricher.

The role of lipid as an energy reserve during the first larval stages of fishes has been reported by many authors (Ehrlich, 1974; Love, 1980; Kanazawa, 1985). Triacylglycerols are considered as the main energy reserve (Hakanson, 1989; Tandler et al., 1989) while phospholipids and even proteins seem to be used when there is an energy deficiency (Kimata, 1983; Fyhn et al., 1987; Tandler et al., 1989).

Planas et al. (1991) reported a decrease in protein (41%), carbohydrate (32%) and lipid (5 1%) in larvae starved from 2 to 5 days. When newly hatched larvae were fed on Selco-enriched rotifers (an emulsion with high levels of lipid), there was a decrease in lipid (triglycerides, sterol esters + waxes, free fatty acids and phospholipids) and protein (Planas et al., 1992a) in the larvae. When the larvae were fed on Protein Selco-enriched rotifers, an increase in protein (31%) and carbohydrate (100%) and a decrease in lipid (2%) were observed between days 2 and 5 posthatching, and between days 5 and 7 both proteins and lipids increased (5 1 and 18%, respectively) (Planas et al., 1991). Therefore, the protein and lipid content of the larval food seems to be an important factor in determining its nutritional value.

Once the larvae have consumed their yolk reserves, they catabolize mainly triglycerides, waxes and phospholipids (Planas et al., 1991). After enrichment with any of the diets tested in this study the rotifers accumulate mainly energetic lipids (triacylglycerols and sterol esters + waxes) and structural lipids.

The suitability of a larval diet is also related to its fatty acid content. Gatesoupe et al. (1977), Léger et al. (1979) and Le Milinaire et al. (1983) have reported dietary requirements of n-3 HUFA of between 0.55 and 1.8% of the dry weight of the food for larval and juvenile turbot. Nevertheless, Gatesoupe and Le Milinaire (1984) reported very low survival rates in turbot larvae after feeding rotifers containing 1.6% n-3 HUFA. Our results show that the n-3 HUFA content in non-enriched rotifers was very low (0.4%) but increased to the required levels after 3 h of enrichment with Super or Protein Selco and after 6 h with Dry Selco. Planas et al. (1991, 1992b) have shown that the fatty acids depleted between days 2 and 5 were mainly the n-3 (n-3 HUFA), n-9 (18:1 n-9) and 16:0. In our experiments, the fatty acid content of the rotifers was enhanced by the enrichment and the increase was particularly clear in the case of n-3 HUFA (20:5n-3 and 22:6n-3).

The requirements for other fatty acids such as 18:3(n-3) and 20:4(n-6) have been studied in juvenile turbot but are unknown for the larvae. Léger et al. (1979) reported a requirement of 1% 18:3(n-3) for turbot juveniles and Linares and Henderson (1991) indicated that turbot juveniles require dietary 20:4(n-6) for incorporation into phosphatidylinositol. The three enrichers tested in our experiments improved the levels of 18:3(n-3) and 20:4(n-6) in the rotifers and the values were highest in the case of Super Selco-enriched rotifers.

Further studies will be carried out in order to verify the effectiveness of these enrichers in larval culture.

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TABLE 1

Proximate composition (% dry weight) of the three enrichers and rotifers before and after enrichment and dry weight (ng/rotifer) of the rotifers [1,2]

Parameter	Initial rotifers	Protein Selco enriched			Dry Selco enriched			Super Selco enriched		
		Enricher	Rotifers 3h	Rotifers 6h	Enricher	Rotifers 3h	Rotifers 6h	Enricher	Rotifers 3h	Rotifers 6h
Protein	36.06±0.52[a]	32.70±1.12	34.54±0.16	38.11±0.97[b]	4.44±0.23	34.14±1.69	37.16±2.06[b]	0.00±0.00	36.16±1.88	39.54±0.67[c]
Carbohydrate	16.65±0.83[a]	33.59±0.90	16.02±1.46	15.50±2.07[b]	1.76±0.02	15.45±1.90	14.20±0.94[c]	0.00±0.00	15.80±1.33	13.03±0.76[d]
Lipid	10.4±10.97[a]	30.29±0.75	13.89±1.50	18.48±1.70[b]	56.14±2.13	14.25±1.80	17.54±2.75[b]	94.20±3.20	15.66±1.80	19.85±1.90[c]
Dry weight	369.5±9.0[a]		419.5±4.5 [c]	453.5±4.8[b]		400.0±7.0	386.0±6.2[a]		330.5±3.0	316.5±3.7

[1]Mean ± s.d. of two replicate groups.

[2]Means within the same rows with different superscript letters are significantly different (P< 0.05)

TABLE 2

Lipid class content (mg/g dry weight) of the three enrichers and rotifers before and after enrichment [1,2]

Lipid class	Initial rotifers	Protein Selco enriched			Dry Selco enriched			Super Selco enriched		
		Enricher	Rotifers 3h	Rotifers 6h	Enricher	Rotifers 3h	Rotifers 6h	Enricher	Rotifers 3h	Rotifers 6h
Phospholipids	48.3.3±0.9[a]	17.9±1.2	65.0±1.3	54.7±1.4[b]	59.4±2.1	63.2±0.5	65.9±0.7[c]	49.4±2.1	57.1±0.9	91.1±1.2[d]

Sterol esters waxes	21.9±1.1[a]	ND	28.6±1.0	25.4±1.0[b]	ND	28.4±0.5	36.1±0.5[c]	ND	39.2±0.6	45.6±1.0[d]
Triacylglycerols	18.7±1.2[a]	32.9±1.1	17.5±1.1	45.6± 0.9[b]	ND	18.1±0.6	38.7±0.4[c]	ND	28.8±0.4	51.1±0.9[d]
Free fatty acids	9.7±0.2[a]	30.6±0.9	14.8±0.2	21.2±0.9[b]	36.7±1.2	20.6±0.7	20.6±0.2[c]	ND	28.7±0.3	42.2±0.1[d]
Sterols	3.7±0.3[a]	ND	2.7±0.1	2.9±0.1[b]	ND	4.1±0.1	4.0±0.1[c]	ND	3.9±0.1	3.7±0.1[d]
Akyl glyceryl ethers	ND	204.0±3.1	ND	ND	411.0±4.6	ND	ND	687.1±5.6	ND	ND

[1]Mean ± s.d. of two replicate groups. ND indicates not detected.

[2]Means within the same row with different superscript letters are significantly different (P<0.05).

TABLE 3

Fatty acid composition (mg/g dry weight) of three enrichers and rotifers before and after enrichment [1,2]

Fatty acid	Initial rotifers	Protein Selco enriched			Dry Selco enriched			Super Selco enriched		
		Enricher	Rotifers 3h	Rotifers 6h	Enricher	Rotifers 3h	Rotifers 6h	Enricher	Rotifers 3h	Rotifers 6h
14:0	0.57±0.01[a]	5.30±0.02	1.70±0.42[b]	1.70±0.04	8.40±0.03	1.20±0.43	l.79±0.23[b]	4.20±0.03	1.05±0.45	1.05±0.96[c]
16:0	4.20±0.18[a]	15.50±0.10	6.10±0.50[b]	6.22±0.10	26.70±0.60	4.62±1.80	6.51±0.88[b]	8.80±0.90	4.17±1.99	4.38±0.90[a]

16:1(n-9)	0.24±0.03[a]	0.00±0.00	0.40±0.01[b]	0.00±0.00	0.00±0.00	0.23±0.33	0.17±0.02[a]	0.00±0.00	0.26±0.37	0.00±0.00[c]
16:1(n-7)	6.65±0.36[a]	7.50±0.20	8.49±0.70[b]	8.26±1.24	9.70±0.90	6.35±2.33	7.74±1.35[c]	0.00±0.00	6.07±1.44	6.08±1.23[a]
17:0	0.20±0.03[a]	1.40±0.01	0.55±0.01[b]	0.47±0.07	1.70±0.01	0.41±0.07	0.53±0.08[b]	2.30±1.01	0.36±0.06	0.40±0.30[c]
17:1(n-7)	0.40±0.01[a]	1.00±0.01	0.62±0.02[b]	0.53±0.14	2.50±0.20	0.41±0.06	0.29±0.04[a]	2.80±0.91	0.26±0.37	0.00±0.00[c]
18:0	2.84±0.13[a]	6.00±0.50	3.60±0.10[b]	3.44±0.58	8.30±0.90	2.87±1.02	3.56±0.39[b]	16.00±0.99	3.29±0.96	4.00±0.99[b]
18:1(n-11)	1.08±0.05[a]	0.00±0.00	1.18±0.05[a]	0.99±0.26	0.00±0.00	1.06±0.41	1.18±0.06[a]	0.00±0.00	1.03±0.55	1.05±0.63[a]
18:1(n-9)	9.53±0.4[a]	14.20±0.70	12.77±1.12[b]	12.49±2.22	22.20±1.10	9.41±2.77	12.10±1.35[b]	27.60±2.34	10.21±2.64	15.90±2.51[c]
18:1(n-7)	1.24±0.09[a]	2.80±0.08	1.55±0.04[a]	1.72±0.18	6.90±0.30	1.32±0.86	1.63±0.13[a]	8.50±1.10	1.65±0.91	2.30±0.56[b]
18:2(n-6)	5.30±0.2[a]	10.60±0.09	6.78±0.17[b]	6.55±1.36	10.90±1.1	05.66±1.79	7.80±1.11[c]	6.10±0.90	5.23±1.92	6.41±1.23[b]
18:3(n-3)	0.36±0.01[a]	1.60±0.05	0.55±0.50[b]	0.52±0.00	2.30±0.07	0.21±0.29	0.61±0.02[bc]	2.50±0.99	0.26±0.36	0.90±0.02[c]
20:1(n-9)	1.29±0.04[a]	5.20±0.01	1.75±0.21[ab]	2.16±0.49	4.70±0.99	1.34±0.37	1.90±0.10[ab]	7.30±1.10	1.64±0.49	2.20±0.99[b]
20:2(n-6)	0.60±0.01[a]	0.00±0.05	0.76±0.37[a]	0.66±0.24	0.00±0.00	0.31±0.44	0.74±0.06a	0.00±0.00	0.59±0.21	0.60±0.32[a]

20:3(n-6)	1.00±0.03[a]	0.00±0.00	1.08±0.10[a]	0.93±0.34	0.00±0.00	1.02±0.29	1.04±0.19[a]	0.00±0.00	1.15±0.16	0.80±0.23[a]
20:4(n-6)	0.58±0.02[a]	2.70±0.04	0.81±1.04[a]	0.93±0.04	0.00±0.00	0.63±0.25	0.88±0.55[a]	7.30±1.26	0.95±0.54	1.40±0.05[b]
20:S(n-3)	2.25±0.13[a]	35.10±0.70	6.28±1.04[b]	8.20±0.99c	57.40±2.00	4.27±1.89	8.38±0.06[c]	96.70±3.20	6.90±0.99	13.80±1.23[d]
22:1(n-9)	0.33±0.01[a]	0.00±0.00	0.73±0.41[b]	0.49±0.30	0.00±0.00	0.26±0.06	0.59±0.06[b]	3.90±1.30	0.31±0.43	0.40±0.03[a]
22:2(n-6)	0.33±0.05[a]	0.00±0.00	0.00±0.00[b]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00[b]	0.00±0.00	0.21±0.03	0.00±0.00[b]
22:5(n-3)	0.85±0.04[a]	5.10±0.20	1.71±0.13[b]	1.96±0.16	7.40±0.05	1.22±0.00	1.77±0.20[b]	16.40±1.56	1.53±0.97	2.90±0.36[c]
22:6(n-3)	1.31±0.03[a]	34.70±0.90	5.22±0.11[ab]	6.52±0.26	54.60±2.10	2.90±0.22	5.59±0.71[b]	114.60±2.30	6.67±2.36	12.80±2.96[c]
24:1(n-9)	0.55±0.01[a]	1.20±0.09	0.56±0.16[a]	0.81±0.50	2.90±0.30	0.27±0.06	0.71±0.10[a]	4.30±1.20	0.32±0.45	0.70±0.01[a]
Σ Saturated	7.81±0.32[a]	28.30±0.51	11.95±0.51[b]	11.83±0.59	45.10±1.08	9.10±2.11	12.39±0.99[c]	31.30±1.68	8.87±2.25	9.83±1.67[d]
Σ Monoenes	21.31±1.06[a]	31.90±0.74	28.05±1.40[b]	27.45±2.65	48.90±1.79	20.65±3.78	26.3±1.92[c]	54.40±3.44	21.75±3.32	28.63±3.08[b]
Σ	12.58±0.49[a]	89.80±1.16	23.19±1.62[b]	26.27±1.75	132.60±3.10	16.22±2.69	26.81±1.45[c]	243.60±4.61	23.49±3.41	39.61±3.47[d]

Polyunsat.										
Σn-6	7.81±0.30[a]	13.30±0.11	9.43±1.12[b]	9.07±1.42	10.90±1.10	7.62±1.88	10.46±1.25[c]	13.40±1.54	8.13±2.01	9.21±1.29[b]
n-3 HUFA	4.41±0.21[a]	74.90±1.14	13.21±1.05[b]	16.68±1.03	119.40±2.00	8.39±1.90	15.74±0.21[c]	227.70±4.23	15.10±2.74	29.50±3.22[d]
n-3 ± n-6	0.61±0.01[a]	5.76±0.13	1.46±0.12[b]	1.90±0.29	11.17±0.10	1.13±0.22	l.56±0.12[b]	17.18±0.10	1.89±0.45	3.30±0.33[c]

[1]Mean ± s.d. of two replicate groups.

[2]Means within the same rows with different superscript letters are significantly different ($P<0.05$).

TABLE 4

Caloric content of the rotifers (Cal/10^3 rotifers) before and after enrichment1

Treatment	Protein	Carbohydrate	Lipid	Total
0h	0.75	0.25	0.34	1.34
3h				
Protein Selco	0.82	0.28	0.50	1.60
Dry Selco	0.77	0.25	0.49	1.51
Super Selco	0.68	0.21	0.45	1.34
6h				

Protein Selco	0.98	0.29	0.73	2.00
Dry Selco	0.81	0.22	0.59	1.62
Super Selco	0.71	0.17	0.54	1.42

[1]The conversion factors used to calculate these values were 5.65, 4.10 and 8.66 cal/mg for protein, carbohydrate, and lipid, respectively (Brett and Groves, 1979).