JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 23, No. 1
March, 1992

Enhancement of the Fatty Acid Composition of the Nematode *Panagrellus redivivus* Using Three Different Media[1]

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Abstract

The free living nematode *Panagrellus redivivus* has shown promise as a live food for marine shrimp and fish. This study evaluated the effect of three media on the fatty acid (FA) profile of nematodes. The media tested were: wheat flour (WF), wheat flour plus yeast (WFY), and wheat flour plus yeast plus fish oil (WFFO).

The total lipid content of nematodes grown on WFFO (14.9%) was significantly higher than the lipid content of nematodes grown on WF (2.7%) and WFY (4.4%). The lipid of nematodes grown on WFFO also contained a higher percentage of n-3 highly unsaturated fatty acids (HUFA) (11.2%), especially 20:5(n-3) (7.4%) and 22:6(n-3) (3.3%), than nematodes grown on WF (4.8%) and WFY (5.7%). Nematodes grown on WFFO medium had similar lipids as *Artemia*, especially desirable n-3 HUFA's.

Total daily yield of nematodes was highest on WFY. There was no difference in yields of nematodes on WF and WFFO. This study has shown that the nutritional value of nematodes can be influenced by the media on which they are cultured. With a proper medium, cultured nematodes can provide the inexpensive, consistently nutritious live food that can be considered for larval culture of marine crustaceans and fish.

Live foods are required by larvae of many commercially important marine fish and crustaceans. Prepared artificial diets are not always accepted and, if accepted, may not be assimilated (Baragi and Lovell 1986). Brine shrimp nauplii, *Artemia* sp., comprise 85% or more of the live foods fed to larval fishes worldwide (Sorgeloos 1980). Reasons for the widespread use of *Artemia* are primarily because the cysts are easily stored and hatched, and the hatched nauplii are readily consumed and assimilated by most larvae (Sorgeloos 1980). However, the nutritional quality among sources of *Artemia* is inconsistent (Sorgeloos 1980; Webster 1989), they are too large for some early stage larvae to eat (Mock et al. 1980), they consume diatoms and other larval food in culture tanks (Biedenbach et al. 1989) and they are expensive ($18–30 per 454 g of cysts).

An alternative food that has shown promise as a live food for fish and crustacean larvae is the free-living nematode *Panagrellus redivivus* which has several practical advantages over *Artemia*. They are easily and inexpensively cultured in the laboratory on simple media and are smaller (50 μm dia) than *Artemia* nauplii (200–225 μm) (Mock et al. 1980; Fontaine et al. 1982; Wilkenfield et al. 1984). Nematodes have a high protein content (48%) with a similar amino acid composition to that of *Artemia* (Kahan et al. 1980). *P. redivivus* have been used as a larval food for common carp and silver carp fry (Kahan et al. 1980) and for marine shrimp (Sebastian and Marion 1969; Fontaine et al. 1982; Wilkenfield et al. 1984; Biedenbach et al. 1989).

Nematodes are most commonly cultured on a moist paste made from either cornmeal, oatmeal, or wheat flour (Watanabe et al. 1980; Fontaine et al. 1982; Radwin 1988;

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Biedenbach et al. 1989). The medium on which nematodes are cultured has been shown to have a significant effect on nematode yield (Radwin and Rouse 1990). It would seem reasonable to expect the medium to affect the biochemical composition and, therefore, nutritional value of the nematodes. Biochemical analysis of nematodes grown on wheat media or cornmeal and wheat media showed that n-6 fatty acids predominated (Radwin 1988; Biedenbach et al. 1989). Watanabe et al. (1980) contend that many marine larvae require n-3 highly unsaturated fatty acids (HUFA) for good growth and survival. The purpose of this study was to evaluate the effect of three different culture media on the fatty acid profile of the nematode *Panagrellus redivivus*.

Materials and Methods

Culture Media

The three culture media evaluated were wheat flour (WF), wheat flour + yeast (WFY), and wheat flour + yeast + fish oil (WFFO). Previous studies have shown wheat flour to provide good yields of nematodes and it was chosen as the standard medium in this study. The standard medium was prepared by making a moist paste from 50–60 ml water and 50 g of wheat flour. The yeast solution for treatments 2 and 3 was prepared by dissolving 5 g baker's yeast in 65 ml of water and adding 5 ml to the wheat flour medium every 7 d. The fish oil for treatment 3 was prepared by mixing 15 g of chicken egg yolk with 60 ml of cod liver oil and 10 ml was poured over the wheat flour medium every 7 d.

All trials were conducted in square, plastic containers with an area of 100 cm^2. Nine containers were assigned randomly to the three treatments, each with three replicates. After preparing the culture containers, each was inoculated with 30,000 live nematodes. Inoculation was accomplished by mixing a freshly harvested sample of nematodes in a known volume of water. The density of worms was estimated by counting five 1 ml aliquots of the mixture. Each culture container was then inoculated with the same volume of water-nematode mixture.

A fine mesh cloth was fastened over each container during culture to prevent contamination from insects. Culture media were kept moist by daily additions of 5–10 ml of water. Containers were maintained in a well ventilated closet at 20 to 24 C. Worms were harvested every 24 h with a spatula by removing the worms that had crawled up the container walls above the media. All worms were weighed to the nearest milligram to estimate daily yield.

Lipid Analysis

Nematodes were collected on nylon mesh, rinsed with water, blotted dry, and placed into a clean, screwcap (Teflon lined) test tube. The lipid fraction was extracted from the nematodes essentially as described by Kates (1986). The small size of the nematodes insured complete extraction without homogenization (Weete et al. 1983; Kates 1986). The samples received 10 ml methanol/chloroform (2:1; v/v) containing 0.5% water and were extracted for 1.5 h with periodic manual shaking. Partially extracted nematodes were collected by centrifugation (130 × gravity for 10 min) and the extraction procedure was repeated two more times with methanol/chloroform (1:1; v/v) for 1 h. Lipids were washed two times with 4 ml of a 6% NaCl solution and dried under nitrogen in a water bath (27 C). The extracted lipid fraction was stored under nitrogen and frozen (−20 C).

Fatty acids (FA) and sterols were separated by alkaline hydrolysis (0.4 ml 33% KOH in 4 ml 95% ethanol) for 120 min at 90 C (Kates 1986). The nonsaponifiable fraction, containing sterols, was obtained by washing the hydrolysate three times with 4 ml hexane. The nonsaponifiable fraction was removed, dried under nitrogen, and stored (−20 C). The hydrolysate was then acidified (pH 1–2) with 6 N HCl, and the saponifiable fraction, containing FA, was obtained by washing three times with 4 ml hexane. FA

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were converted to their methyl ester derivatives using 12% BF_3 in methanol (Morrison and Smith 1964).

FA methyl esters were analyzed with a Hewlett-Packard 5710A gas chromatograph (Hewlett-Packard, Avondale, Pennsylvania) equipped with a flame-ionization detector and a 30 m glass capillary column (Supelco, Inc., Bellefonte, Pennsylvania) coated with SP-2330. The carrier gas was nitrogen with a column pressure of 0.575 kg/cm^2. Injector and detector temperatures were both 300 C and the oven temperature was programmed from 140 to 210 C at 4 C/min. Detector response was recorded and quantitated with a Hewlett-Packard 3380A integrator-recorder. Methyl esters were identified by comparison with those of standards (Applied Science Laboratories, Deerfield, Illinois; Nu Chek Prep, Inc., Elysian, Minnesota). An internal standard (24:1n-9, nervonic acid) was added to the samples prior to hydrolysis.

Sterols were acetylated with 0.25 ml pyridine and 0.25 ml acetic anhydride and heated at 60 C for 15 min (Kates 1986) and analyzed using a Hewlett-Packard 5710A gas chromatograph equipped with a 30 m capillary column coated with DB-5 (J & W Scientific, Folsom, California). Carrier gas was helium with a column pressure of 1.0 kg/cm^2. Injector and detector temperatures were both 300 C and the oven temperature was isothermic at 280 C. The detector response was recorded and quantitated with a Hewlett-Packard 3380A integrator-recorder. The internal standard was 5-dihydro-cholesterol (Sigma Chemical Co., St. Louis, Missouri) and sterols were identified by comparison to sterol standards (Sigma Chemical Co.).

Statistical Methods

Analysis of variance (ANOVA) was computed on the total nematode yield, percentage of total lipid, FA percentages, and the amount of cholesterol using the SAS ANOVA procedure (Statistical Analysis Systems 1985). Total lipid and FA percentages were transformed to arcsin values prior to analysis (Zar 1984). Tukey's test was used to determine where differences existed among treatment means. Untransformed data are reported to facilitate comparison with results from other related studies.

Results

WFY gave a significantly higher daily yield than WF or WFFO during the five-week study ($P < 0.01$) (Fig. 1). There was no difference in yields between WF and WFFO ($P < 0.05$). Total yield for WFY was 51.3 mg nematodes/cm^2, while yields of WF and WFFO were 27.4 and 25.5 mg/cm^2, respectively.

Nematodes grown on the WFFO medium had a significantly higher percentage of total lipid (14.9%) than nematodes cultured on WF (2.7%) and WFY (4.4%) (Table 1). Cholesterol was the only sterol present and was not significantly different among treatments ($P > 0.05$). The fatty acid content of nematodes grown on the three media showed variation. Nematodes grown on WF and WFY had a significantly higher percentage of 18:2(n-6) (linoleic acid) and lower percentages of the (n-3) HUFA 18:3(n-3) (linolenic acid), 20:5(n-3) (eicosapentaenoic acid or EPA), and 22:6(n-3) (docosahexaenoic acid or DHA) than those grown on WFFO ($P < 0.05$) (Table 2).

The majority of lipid in nematodes was unsaturated FA, with 74.3, 76.7, and 76.4% of the total FA being unsaturated for nematodes grown on WF, WFY, and WFFO media, respectively. Monoenoic acids comprised a significantly higher percentage in nematodes grown on WFFO (40.5%) than in those grown on WF and WFY (29.6 and 34.2%, respectively) due to a higher percentage of 16:1(n-7) (palmitoleic acid) ($P < 0.05$). Dieneoic acids were significantly higher ($P < 0.05$) in nematodes grown on WF and WFY because of the large percentage of linoleic acid. Polyenoic acids were not significantly different ($P > 0.05$) among treatments; however, total percentage of n-3

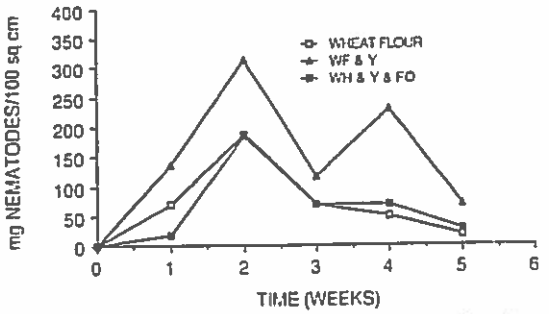

FIGURE 1. *Weekly yield of live nematodes (mg/100 cm² day) cultured on three media. Media were wheat flour, wheat flour plus weekly applications of yeast (WF + Y) and wheat flour plus weekly applications of yeast and fish oil (WF + Y + FO).*

fatty acids was significantly higher in nematodes grown on WFFO than those grown on WF and WFY ($P < 0.05$).

Discussion

Production Characteristics

Yields obtained on all media in this study were similar to those obtained by Radwin and Rouse (1990) on wheat flour. Yields peaked around the second week and then began to decrease over the following three weeks. The addition of fish oil to the media seemed to suppress nematode yields slightly the first week, but by the second week, yields on the WF and WFFO were similar and remained so during the remainder of the study. The WFY gave the highest yields.

TABLE 1. *Percentage of total lipid and amount of cholesterol (μg/mg nematode dry wt.) in nematodes grown on three media.*[a]

Media[b]	Total lipid (%)	Cholesterol (μg/mg dry wt.)
WF	2.73 ± 0.34b	1.97 ± 0.10a
WFY	4.44 ± 0.34b	4.10 ± 1.59a
WFFO	14.87 ± 2.77a	0.17 ± 0.05a

[a] Values are means ± SE of 2 replications. Means with the same letter are not significantly different for vertical comparisons ($P > 0.05$).

[b] Media used were wheat flour (WF), wheat flour + yeast (WFY), and wheat flour + yeast + fish oil (WFFO).

Lipid Analysis

Lipid analysis of the nematodes cultured on the various media showed that the culture medium can produce marked differences in FA content of nematodes. The total lipid content of *P. redivivus* grown on WFFO medium was significantly higher than the lipid content of nematodes grown on WF and WFY. Since the nematodes were rinsed thoroughly prior to lipid extraction, the higher percentage of total lipid from the nematodes should be due to consumption of the medium and assimilation of the lipids by the nematodes. The total lipid of the nematodes from WFFO (14.9%) was comparable to total lipid in *Artemia* from China (15.0%) and the Great Salt Lake (16.2%) (Webster 1989). Nematodes grown on WFFO had a higher percentage of EPA than *Artemia* from the Great Salt Lake and was close to that found in a source from San Francisco Bay (Table 3). Nematodes also had DHA which was not found in either *Artemia* source.

There is strong evidence that n-3 HUFA, especially EPA and DHA, are important in larval nutrition (Watanabe et al. 1978). Watanabe et al. (1978) stated that differences in growth and survival in red seabream *Pagrus major* were due to the variation in n-3 HUFA, with higher percentages of n-3 fatty acids producing higher growth and survival rates. *Artemia* with a high percentage of EPA have been found to provide greater growth and survival in larval red seabream (Watanabe et al. 1980) and striped bass *Morone saxatilis* (Webster 1989) than fish fed with a lower percentage of EPA. Jones et al. (1979) reported that penaeid larvae have a requirement for EPA and DHA. Other crustaceans, such as the mud crab *Eurypanopeus depressus* were also found to require a dietary source of EPA, and possibly DHA, for good growth and survival (Levine and Sulkin 1984).

Only traces of DHA have been found in brine shrimp nauplii (Fujita et al. 1980; Webster 1989). Hinchcliffe and Riley (1972)

TABLE 2. *Percentage of fatty acids (wt. %) in the total lipids extracted from nematodes grown on three media.*[a]

Fatty acid	Media[b]		
	WF	WFY	WFFO
12:0	0.40 ± 0.40	0.30 ± 0.12	0.20 ± 0.20
14:0	2.73 ± 0.02b	3.91 ± 0.22a	4.67 ± 0.13a
14:1(n-5)	0.19 ± 0.07	0.05 ± 0.05	1.52 ± 0.69
16:0	11.05 ± 0.51a	7.80 ± 0.03b	12.89 ± 0.58a
16:1(n-7)	4.71 ± 0.13b	4.72 ± 0.15b	10.46 ± 0.70a
17:0	0.89 ± 0.06	0.48 ± 0.34	0.42 ± 0.03
18:0	7.58 ± 0.83ab	8.19 ± 0.48a	4.70 ± 0.27b
18:1(n-9)	8.42 ± 0.16b	7.40 ± 0.19b	15.05 ± 2.04a
18:1(n-7)	11.15 ± 0.59b	15.69 ± 0.39a	11.28 ± 0.55b
18:2(n-6)	28.38 ± 0.47a	25.17 ± 0.88a	9.91 ± 1.70b
18:3(n-3)	5.03 ± 0.29	3.23 ± 0.10	9.28 ± 2.32
20:0	1.29 ± 0.02a	1.38 ± 0.04a	0.23 ± 0.02b
20:1(n-9)	0.50 ± 0.04	0.23 ± 0.07	1.02 ± 0.23
20:3(n-3)	0.09 ± 0.09	1.06 ± 1.06	0.44 ± 0.44
20:4(n-6)	6.37 ± 0.32ab	8.23 ± 0.39a	4.64 ± 0.35b
20:5(n-3)	4.56 ± 0.34b	4.61 ± 0.21b	7.35 ± 0.59a
22:0	1.80 ± 0.47	1.24 ± 0.92	0.47 ± 0.04
22:1(n-9)	3.98 ± 0.37a	5.14 ± 0.05a	1.52 ± 0.04b
22:2(n-6)	0.11 ± 0.11b	0.09 ± 0.09b	0.78 ± 0.06a
22:4(n-6)	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.08
22:5(n-3)	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11
22:6(n-3)	0.15 ± 0.15b	0.00 ± 0.00b	3.25 ± 0.70a
% saturates	25.72 ± 1.31	23.28 ± 0.67	23.56 ± 0.04
% monoenes	29.56 ± 0.17b	34.20 ± 0.23b	40.50 ± 1.29a
% dienes	26.99 ± 0.92a	25.26 ± 0.79a	10.69 ± 1.76b
% polyenes	16.22 ± 0.60	17.12 ± 1.56	25.12 ± 3.02
% n-3	9.86 ± 0.29b	8.89 ± 1.17b	20.41 ± 2.75a
% n-6	34.86 ± 0.90a	33.49 ± 0.58a	15.41 ± 1.50b
n-3/n-6	0.28 ± 0.0b	0.26 ± 0.01b	1.34 ± 0.13a

[a] Values are means ± SE of 2 replications. Means with different letters are significantly different for horizontal comparisons ($P < 0.05$).

[b] Media used were wheat flour (WF), wheat flour + yeast (WFY), and wheat flour + yeast + fish oil (WFFO).

found no DHA in brine shrimp nauplii even when fed algae with a high percentage of DHA. The lipid of nematodes grown on WFFO contained a higher percentage of n-3 HUFA (11.15%), especially EPA (7.35%) and DHA (3.25%), than nematodes grown on WF and WFY media. This latter finding may explain in part why Sivapalan and Jenkins (1966) and Fontaine et al. (1982) did not obtain as good survival and growth of penaeid larvae fed nematodes cultured on cornmeal or oatmeal media, both low in EPA and DHA. Biedenbach et al. (1989) obtained good growth and survival in *Penaeus vannamei* by feeding high numbers of nematodes; however, their rearing units also contained diatoms, which could have supplemented the dietary n-3 HUFA.

Penaeid larvae may have different fatty acid requirements. Ward et al. (1979) stated that *P. setiferus* required the n-6 HUFA 20:4 (arachidonic acid) in the diet. Biedenbach et al. (1989) found that feeding nematodes with a high n-6 fatty acid content to larval *P. vannamei* resulted in similar growth to larvae fed *Artemia* nauplii with a high percentage of EPA; however, larvae were also fed diatoms which have EPA. *P. japonicus* larvae are also reported to require dietary EPA and DHA for good growth and survival (Jones et al. 1979).

This study has shown that the nutritional

TABLE 3. *Percentage of fatty acids (wt.) in total lipids extracted from nematodes cultured on wheat flour media with fish oil and two strains of* Artemia.

Fatty acid	Nematode (WFFO)	*Artemia* (GSL)[a]	*Artemia* (SFB)[a]
12:0	0.2	0.26	0.08
14:0	4.67	0.78	1.24
14:1(n-5)	1.52	0.98	0.36
16:0	12.89	14.12	11.11
16:1(n-7)	10.46	20.52	3.34
17:0	0.42	1.11	1.67
18:0	4.7	7.51	4.07
18:1(n-7)	11.28	8.78	7.70
18:2(n-6)	9.91	8.23	4.78
18:3(n-3)	9.28	28.19	3.76
20:0	0.23	0.63	0.6
20:1(n-9)	1.02	0.58	0.56
20:3(n-3)	0.44	0.27	0.34
20:4(n-6)	4.64	1.72	2.15
20:5(n-3)	7.35	1.19	9.32
22:0	0.47	0.27	0.04
22:1(n-9)	1.52	0.12	0.10
22:2(n-6)	0.78	0.36	0.12
22:4(n-6)	0.08	0.01	0.01
22:5(n-3)	0.11		
22:6(n-3)	3.25		
% (n-3)	20.41	13.44	29.68
% Saturates	23.56	22.13	22.54

[a] From Webster (1989).

value of nematodes can be altered by the media on which they are cultured. Supplementation of the FA content of nematodes, especially the n-3 HUFA EPA and DHA, may allow the use of nematodes as the only food for some marine fish and shrimp larvae. By controlling the culture medium, a more nutritionally constant live food can be produced.

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