. The effects of coconut oil on reproductive traits and fatty acid eggcomposition of rainbow Oncorhynchus mykiss, Walbaum)

Aquaculture International **11:** 289–299, 2003.

The effect of dietary coconut oil on reproductive traits and egg fatty acid composition in rainbow trout (*Oncorhynchus mykiss*)

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Received 28 August 2001; accepted in revised form 4 March 2003

Key words: Coconut oil, Eggs, Fatty acids, *Oncorhynchus mykiss*, Rainbow trout, Reproduction

Abstract. Two hundred and twenty rainbow trouts (IBW: 700 g) were randomly allotted to four tanks, with a male/female ratio of 0.56. Fish were fed for 168 d with four experimental diets containing herring oil, cod liver oil and coconut oil with the following inclusion rates: diet A: 12-1-0% respectively; diet B: 6-1-6%; diet C: 0-1-12%; diet D: 0-0-13%. Irrespective of the dietary treatment, weight gains of broodstocks were high (> 3 g/d) and FCR below 2. No significant difference was observed concerning the total amount of eggs spawn, egg average weight (82.5 mg/egg) and lipid content (5.4 mg/egg). However, the fatty acid profile of eggs was significantly affected by the dietary treatments. The content of unsaturated fatty acids, particularly the n-3 fatty acid series (EPA and DHA) significantly decreased with increasing levels of coconut oil in the diet.

Introduction

Several factors affect egg and fry quality in fish species. These are either endogenous: genotype, age and size of broodstock, ovarian characteristics, egg size and gamete age (Craik and Harvey 1984; Knox et al. 1988; Lahnsteiner et al. 1999; Carillo et al. 2000) or exogenous: bacterial colonisation of egg surface, egg management, broodstock feeding (Bromage and Roberts 1995). In particular, reproductive performance is deeply affected by the nutritional status of fish, which is known to condition several reproductive traits, such as age at maturity, fecundity, egg size, chemical composition of eggs and also embryonic development (Shepherd and Bromage 1988; Eskelinen 1989; Carillo et al. 2000). In freshwater fish, embryonic development depends on the energetic reserves of the yolk sac. This is particularly true for Salmonids, as their embryonic structures are highly developed and their trophic function lasts for many days after hatching. Previous research demonstrated that changes in yolk composition, through diet and feeding levels, could also affect fry survival (Springate et al. 1985; Knox et al. 1988). Among dietary components a primary role is played by energy. In fact net energy requirements for maintenance

are to be satisfied before growth and reproduction (Cho and Kaushik 1990). These authors, and others (Da Silva and Anderson 1995), pointed out that energy may be diverted from somatic growth when energy requirements for reproduction increase at the end of gonadal development.

Maternal nutrition is the only source of fatty acids until the beginning of exogenous feeding. Watanabe et al. (1984) observed that lipid sources in broodstock diets affect egg quality, their hatching rate, also demonstrating that a dietary fatty acid deficiency could cause a decrease in gamete numbers and a high mortality of embryos. On the other hand, broodstock diets rich in polyunsaturated fatty acids (PUFA) increase the risks of eggs being exposed to peroxidation and tissue damage. As a consequence, PUFA level and the presence of antioxidant substances in the gamete are strictly related to egg quality (Pettersson and Lignell 1998; Pickova et al. 1999). Almansa et al. (1999) demonstrated in marine fish that a very long period of feeding with a low quantity of n-3 PUFA can alter egg quality, fatty acid composition of eggs and fry growth, causing anomalies in the development of the nervous tissues. In particular, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) seem to be necessary for the development of eyes and brain of the early stages of fish (Brodtkorb et al. 1997). On the other side, there is growing interest by feed manufacturers in using vegetable or plant lipid sources as replacers of fish oils in diets for finfish, considering that world fisheries are overexploiting natural fish stocks (FAO 2000) and fish oils could become limiting ingredients for the manufacture of animal feeds. Coconut oil is a common plant lipid source with a particular fatty acid profile, being rich in lauric acid, poor in oleic and linoleic acids and with no linolenic acid (Sheppard et al. 1979; Gunstone 1997).

The aim of this trial was to evaluate the effects of four isolipidic and isoproteic diets, through the progressive replacement of fish oils (i.e., polyunsaturated fatty acids), with dietary coconut oil (i.e., saturated fatty acids), on reproductive traits of rainbow trout (RB trout) and on the fatty acid composition and weight of its eggs.

Material and methods

Two hundred and twenty rainbow trouts (2+), from the same population (2n) were randomly distributed into 4 tanks, with a male/female ratio of around 0.56. Well water, characterised by a temperature of 12.5 ± 0.1 °C and dissolved oxygen levels of 7.2 ± 0.4 mg l^{-1}, was provided at a flow rate of 0.7 l sec^{-1} $tank^{-1}$. Four isoproteic diets (49.4% crude protein on DM basis) with constant lipid levels (18% DM) were formulated (Table 1). No ingredient derived from terrestrial animals was used for manufacturing the experimental diets. The dietary lipid composition was changed by modifying the relative proportions of various lipid sources. Herring oil, cod-liver oil and coconut oil were respectively included in the diet with the following inclusion rate: diet A = 12:1:0%; diet B = 6:1:6%; diet C = 0:1:12% and diet D = 0:0:13%. The proximate composition of the diets was determined by the AOAC methods (1995) (Table 1). For 168 days fish were fed by hand, once a day, till ap-

parent satiation and weighed every 21 days. Three days before spawning (second half of November) fish feeding was stopped. Growth performance of RB trout was determined according to the following variables:

$$\text{Feeding level: } g/100g \quad \text{average live fish weight per tank}$$

$$\text{Specific growth rate } (SGR) = (\ln\text{Final Body Weight} - \ln\text{Initial Body Weight}) \times 100/\text{days}$$

$$\text{Thermal Growth Coeff. } (TGC) = (\sqrt{3}\text{Final Body Weight} - \sqrt{3}\text{Initial Body Weight})/\Sigma(T \times days);$$

where
T = Temperature (°C);
Feed Conversion Ratio (FCR): Feed intake (g)/Fish weight gain (g)
Protein efficiency ratio (PER) = fish weight gain (g)/protein intake (g)
The Gonadosomatic index (GSI) of RB trouts was determined using the following formula:

$$GSI = \text{Stripped eggs}(g) \times 100/\text{Fish weight}(g)$$

Eleven to thirteen females from each treatment were manually stripped and the total egg mass of each female weighed. For each female, immediately after stripping, 3 samples of 20 eggs were used to estimate average individual wet weight of the eggs. Another two samples of 20 eggs from each female were immediately frozen (−23 °C) prior to lipid extraction, according to Folch et al. (1957). The fatty acid composition of the diets and eggs was determined by gas liquid chromatographic separation on fatty acid methyl esters, using a 30 m × 0.32 mm capillary column (Omegawax, Supelco) and flame ionization detection. The operating conditions of the chromatograph were as described by Ballestrazzi and Lanari (1996). Reproductive data and fatty acid composition of the eggs were submitted to one way analysis of variance. The comparison between means was performed with LSD test (Snedecor and Cochan 1982).

Results

Fatty acid composition of diet

The level of saturated fatty acid (C10:0, C12:0, C14:0, C18:0) significantly increased from diet A to diet D, according to the inclusion level of coconut oil in the diets ($P < 0.01$; Table 2). There were significant decreases of monounsaturated and polyunsaturated fatty acids from diet A to diet D and consequently the saturated/unsaturated ratio significantly increased in accordance to the inclusion levels of coconut oil in the diets: from 0.38 (A) to 3.85% (D) ($P < 0.01$, Table 2). The con-

Table 1. Formulation (g/kg diet) and proximate composition (% DM, except moisture %) of the experimental diets fed to RB trout broodstock.

	A	B	C	D
Formulation				
Herring meal (999)	230	230	230	230
Soybean meal	250	250	250	250
Soybean Prot. Conc.	85	85	85	85
Corn gluten meal	120	120	120	120
Brewery yeast	50	50	50	50
Herring oil	120	60	0	0
Cod-liver oil	10	10	10	0
Coconut oil	0	60	120	130
Wheat Starch	109.5	109.5	109.5	109.5
Vitamin premix[1]	5	5	5	5
Mineral premix[2]	5	5	5	5
Choline	2.5	2.5	2.5	2.5
Lysine	3	3	3	3
CMC[3]	10	10	10	10
Proximate composition				
Moisture	7.7	7.7	8.2	8.0
Crude protein	48.6	49.3	49.3	50.3
Crude fat	18.3	17.7	18.0	18.0
Ash	6.6	6.6	6.7	6.7
Crude fiber	2.5	2.7	2.9	2.2
N-free extract	24.0	23.7	23.1	22.8

[1] Vitamin premix (except where units are given, values are in g/Kg premix): vit. A, (1.200.000 UI); vit. D3, (250.000 UI); vit. E, 30.0; vit. K, 3.2; thiamin, 3.0; riboflavin, 4.0; pyridoxine HCl, 3.0; Ca-d-panthotenate, 14.0; niacin, 30.0; biotin, 0.2; folic acid, 1.0; vit. B12, 0.01; vit. C, 40.0; inositol, 70.0.
[2] Mineral premix (data expressed in g/Kg premix): Mg, 100; Na, 100; Fe, 12; Zn, 10.0; Cu 1.0; I, 0.6; Se, 0.04; ethoxyquin, 15.0; dextrin to 1000.0.
[3] CMC: carboxymethylcellulose

centration of n-9 and n-3 fatty acids were significantly affected by the dietary treatments, decreasing from A to D (n-9 from 25.51 to 9.98% and n-3 from 28.9 to 4.1%). The levels of n-3 series were mainly provided by EPA and DHA, even in diets C and D, in which no fish oil was included. On the other hand, a slight reduction was noted for n-6 (from 6.05 to 5.24%). Because of this reason the n-3/n-6 dietary ratio significantly decreased: from 4.78 in A to 0.78 in D ($P < 0.01$). Also the DHA/EPA ratio increased from 1.03 in A to 1.69 in D ($P < 0.01$; Table 2).

Productive performance

No loss of appetite or diet refusal behaviour were observed which is indirectly confirmed by the actual feeding levels ($\sim 1\%$ body weight) (Table 3). Two weeks be-

Table 2. Contents of selected fatty acids, main fatty acids classes (% total area) and ratios of the experimental diets fed to RB trout broodstock.

	A	B	C	D	S.E.M. (12 df)
10:0	0.00^{C}	2.67^{B}	5.00^{A}	5.37^{A}	0.2002
12:0	0.00^{D}	21.65^{C}	40.05^{B}	42.56^{A}	0.5810
14:0	6.92^{C}	11.89^{B}	16.15^{A}	16.74^{A}	0.4556
16:0	18.64^{A}	15.00^{B}	12.08^{C}	11.99^{C}	0.2965
16:1 n-7	9.30^{A}	5.16^{B}	1.86^{C}	0.95^{C}	0.1233
18:0	2.06^{B}	2.32^{AB}	2.59^{A}	2.72^{A}	0.1628
18:1 n-9	10.31^{A}	9.71^{AB}	9.14^{B}	8.32^{C}	0.2055
18:1 n-7	2.64^{A}	1.88^{AB}	0.86^{BC}	0.33^{C}	0.4163
18:2 n-6	6.05^{A}	5.74^{AB}	5.34^{B}	5.24^{B}	0.1510
18:3 n-3	1.42^{A}	0.96^{B}	0.55^{C}	0.50^{C}	0.0744
18:4 n-3	3.06^{A}	1.51^{B}	0.00^{C}	0.00^{C}	0.0639
20:1 n-9	6.08^{A}	3.38^{B}	1.24^{C}	0.68^{D}	0.1342
20:4 n-6	0.00	0.00	0.00	0.00	–
20:5 n-3	11.60^{A}	6.36^{B}	1.39^{C}	1.34^{C}	0.3905
22:1 n-9	9.11^{A}	5.08^{B}	1.23^{C}	0.98^{D}	0.2366
22:5 n-3	0.91^{A}	0.00^{B}	0.00^{B}	0.00^{B}	0.0158
22:6 n-3	11.90^{A}	6.67^{B}	2.53^{C}	2.26^{C}	0.1830
SFA[1]	27.62^{D}	53.53^{C}	75.87^{B}	79.38^{A}	0.6886
MUFA[2]	37.44^{A}	25.22^{B}	14.33^{C}	11.26^{C}	1.0362
PUFA[3]	34.94^{A}	21.24^{B}	9.81^{C}	9.34^{C}	0.4349
Unsaturated	72.38^{A}	46.45^{B}	24.14^{C}	20.60^{D}	0.6886
Sat./Unsat.	0.38^{D}	1.15^{C}	3.14^{B}	3.85^{A}	0.1114
MUFA/PUFA	1.07^{B}	1.19^{B}	1.47^{A}	1.20^{B}	0.0500
n-3 PUFA	28.89^{A}	15.50^{B}	4.47^{C}	4.10^{C}	0.4630
n-6 PUFA	6.05^{A}	5.74^{AB}	5.34^{B}	5.24^{B}	0.1526
n-9 PUFA	25.51^{A}	18.17^{B}	11.61^{C}	9.98^{D}	0.4422
n-3/n-6	4.77^{A}	2.70^{B}	0.84^{C}	0.78^{C}	0.1077
DHA/EPA	1.03^{B}	1.05^{B}	1.84^{A}	1.69^{A}	0.2328

Means in the same row not sharing the same superscript letters are significantly different ($P < 0.01$).
[1] SFA: saturated fatty acids
[2] MUFA: monounsaturated fatty acids
[3] PUFA: polyunsaturated fatty acids

fore egg stripping a general decrease of feed intake was observed in all groups. Mortality was lower than 4% for all tanks. Fish reached a final weight of 1.2 Kg, with an individual weight gain ranging from 500 to 600 g and daily growth rates were higher than 3 g/d (Table 3). TGC was higher than 0.8 (/‰) for all treatments and FCR lower than 2 (Table 3). Furthermore, PER was higher than 1, irrespective of the dietary treatment and the relative large size of experimental fish.

Table 3. Performance of RB trout broodstock fed the experimental diets.

	Diets			
	A	B	C	D
Feeding level (% a. b.w.)	1.00	0.97	1.00	0.99
Mortality (%)	3.57	1.92	0.00	1.81
Initial Body Weight (g)	693.2	709.8	709.8	690.1
Final BodyWeight (g)	1274.7	1316.7	1214.3	1188.6
Daily growth (g fish^{-1})	3.48	3.62	3.00	3.05
TGC(×1000)	0.934	0.956	0.819	0.823
FCR	1.70	1.54	1.94	1.87
PER	1.24	1.24	1.06	1.06

Table 4. Reproductive performance of RB trout broodstock fed the experimental diets.

	Diets				
	A	B	C	D	S.E.M. (45 d.f.)
Specimens (n)	13	12	13	11	
Egg mass weight (g)	197.2	213.9	193.4	181.4	55.9343
GSI (% bw)	15.13	14.70	14.02	15.60	4.1232
Egg weight (mg)	85.44	87.23	78.48	79.42	14.6169
Total lipids (% ww)	6.09	6.40	6.53	6.93	1.0863
Total lipids (mg/egg)	5.32	5.50	5.15	5.46	1.3225

Reproductive performance

Female broodstock spawned after 170–175 days, at the end of the feeding period, regardless of the various dietary treatments.

The weight of the total mass of eggs produced by each female ranged from 181 to 213 g/female and GSI varied from 14 to 15.6% (Table 4). Average wet weight of the eggs ranged from 78 to 85 mg, without significant differences between diets. The increasing trend in the lipid content of the eggs (from 6% diet A to 6.9% diet D) was not confirmed by the absolute lipid contents of the eggs: 5.15–5.5 mg/egg (Table 4).

Fatty acid composition of the eggs

Increasing dietary levels of coconut oil caused a significant increase in saturated fatty acids (C 10:0, C 12:0, C 14:0 and C 18:0) in the eggs (Table 5). Also oleic acid was more concentrated in eggs of fish fed diets C and D than in those fed diet A (Table 5). A similar trend was observed for linoleic acid, particularly in the eggs of female from group C (4 *vs* 3.2%; $P < 0.01$), while higher levels of linolenic acid were observed in eggs from females fed diet D. Among the n-3 fatty acids, the

conservative property of DHA was confirmed. Its concentration in the eggs decreased only 16.3% (from 27.55 in A to 23.05% in D), although in the diets the decrease went from 11.9 (A) to 2.26 (D)%. EPA content in the eggs decreased 68.6% from A to C, while dietary EPA level had dropped from 11.6 (diet A) to 1.39% (diet C) (Tables 2 and 5).

The constancy of monounsaturated fatty acid levels in the egg is evident (almost 25%) and it can be noted that females progressively transferred almost 34% of total lipids to their eggs as polyunsaturated fatty acids, although there was a huge decrease in dietary PUFA (Tables 2 and 5). There was no significant difference in the amount of n-6 fatty acids in the eggs, while n-3 significantly decreased (from 40.35 to 27.66%) according to the dietary levels of coconut oil (Table 5). An opposite, increasing trend of n-9 series in the eggs (from 15 in A to 18.17% in D; $P < 0.01$) was observed, although these fatty acids were progressively decreasing in the diet with increasing inclusion rates of coconut oil. Also in the eggs saturated/unsaturated ratio significantly increased (form 0.38 to 0.64; Table 5). PUFA/monounsaturated and DHA/EPA ratios significantly changed either in diets and in the eggs, but to a larger extent in the eggs (Figures 1 and 2).

Discussion

Fat content in fish egg varies from 1.5 to 10% wet weight (ww) (Kairanta and Ackman 1981). Among different finfish species, Sargent et al. (1989) roughly distinguished those whose egg lipid contents are lower than 4%, between 4 and 7% and those above 7% ww. In this last group, which rainbow trout belongs to, there are usually distinct and separated lipid drops and higher neutral lipid content (> 50% ww; Russel, 1976), which derives from neutral lipids, such as triacylglycerols, waxes or cholesterol esters. The general composition of egg lipid classes is a conservative trait of the species, even if broodstock nutrition affects both percentage lipid content and fatty acid composition. In this trial, lipid levels in the eggs ranged, as a function of diets, from 6.09 to 6.93%, which are lower than 7%, indicated by Sargent et al. (1989) for RB trout. Eggs are usually richer in n-3 PUFA than parental body lipids (Kairanta and Ackman 1981; Tocher and Sargent 1984). Polyunsaturated fatty acids in the eggs are mainly DHA and EPA, with an approximate DHA/EPA ratio of 2:1 (Kairanta and Linko 1984; Tocher and Sargent 1984) and without detectable differences between marine and fresh water species. In this trial, DHA/EPA ratio in the eggs was higher than 3 for broodstocks fed diets containing fish oils, and progressively increased, to the maximum value of 8.6, for the eggs from females fed diets rich in coconut oil. Despite a decreasing trend for EPA and DHA in the diet, there was a selective mechanism of deposition of these fatty acid in the eggs, preferentially for DHA. The eggs of freshwater species generally have a lower content of n-3 PUFA than n-6 PUFA, particularly linoeic and arachidonic acid (AA) (Bolgova et al. 1981; Kairanta and Linko 1984). The present results do not confirm the importance of n-6 fatty acids for rainbow trout. About 6% of die-

Table 5. Contents of selected fatty acids, main fatty acids classes and ratios (% total area) of eggs spawn by RB trout broodstock fed the experimental diets.

	Diets				
	A	B	C	D	S.E.M.(45 df)
10:0	0.01^{C}	0.13^{B}	0.34^{A}	0.32^{A}	0.088
12:0	0.95^{C}	4.01^{B}	6.88^{A}	6.85^{A}	1.217
14:0	4.18^{C}	6.88^{B}	9.59^{A}	9.38^{A}	0.8214
16:0	17.38^{A}	16.32^{B}	15.56^{B}	16.02^{B}	0.8832
16:1 n-7	5.58^{A}	4.74^{AB}	4.64^{B}	4.66^{B}	0.8857
18:0	5.09^{C}	5.41^{BC}	6.23^{AB}	6.40^{A}	0.7723
18:1 n-9	12.50^{B}	14.13^{AB}	15.83^{A}	16.23^{A}	2.5983
18:1 n-7	3.92	3.42	3.90	3.68	0.6045
18:2 n-6	3.24^{B}	3.46^{AB}	4.00^{A}	3.73^{AB}	0.6013
18:3 n-3	0.60^{B}	0.49^{B}	0.23^{B}	3.73^{A}	0.4439
18:4 n-3	0.61^{A}	0.33^{B}	0.21^{B}	0.14^{B}	0.1901
20:1 n-9	2.83^{A}	1.95^{B}	2.33^{AB}	1.94^{B}	0.5565
20:4 n-6	0.95^{A}	0.70^{A}	0.27^{B}	0.24^{B}	0.2417
20:5 n-3	8.80^{A}	5.77^{B}	2.76^{C}	3.14^{C}	0.8079
22:1 n-9	0.20^{A}	0.19^{A}	0.02^{B}	0.00^{B}	0.0775
22:4 n-6	0.19^{AB}	0.24^{A}	0.14^{B}	0.14^{B}	0.0632
22:5 n-3	2.79^{A}	1.87^{B}	0.97^{C}	1.10^{C}	0.3379
22:6 n-3	27.55^{A}	27.56^{A}	23.42^{B}	23.05^{B}	3.7437
SFA[1]	27.61^{C}	32.76^{B}	38.60^{A}	38.97^{A}	1.7435
MUFA[2]	25.03	24.44	26.74	26.51	3.2496
PUFA[3]	47.36^{A}	42.81^{B}	34.66^{C}	34.52^{C}	3.9472
Unsaturated	72.39^{A}	67.25^{B}	61.40^{C}	61.03^{C}	1.7436
Sat./Unsat.	0.3822^{C}	0.4878^{B}	0.6296^{A}	0.6400^{A}	4.1437
MUFA/PUFA	0.55^{B}	0.57^{B}	0.78^{A}	0.79^{A}	0.1450
n-3 PUFA	40.35^{A}	36.02^{A}	27.60^{B}	27.66^{B}	4.2391
n-6 PUFA	6.55	6.31	6.87	6.72	0.9950
n-9 PUFA	15.33^{B}	16.09^{AB}	18.15^{A}	18.17^{A}	2.5000
n-3/n-6	6.69^{A}	5.74^{A}	4.04^{B}	4.17^{AB}	1.4832
DHA/EPA	3.15^{C}	4.87^{B}	8.61^{A}	7.59^{A}	0.9434
Arachid/(EPA + DHA)*100	2.73^{A}	2.09^{A}	1.08^{B}	0.94^{B}	0.0077

Means in the same row not sharing the same superscript letters are significantly different. A, B, C: $P < 0.01$

[1] SFA: saturated fatty acids

[2] MUFA: monounsaturated fatty acids

[3] PUFA: polyunsaturated fatty acids

tary fatty acids are from n-6 series and similar levels are found in the eggs, mainly due to arachidonic acid, from maternal source (mobilisation as such) or by elongation and desaturation of C 18:2 n-6 from dietary origin. In mammals, arachidonic acid is the main precursor of prostaglandins, thromboxanes and leukotrienes. These

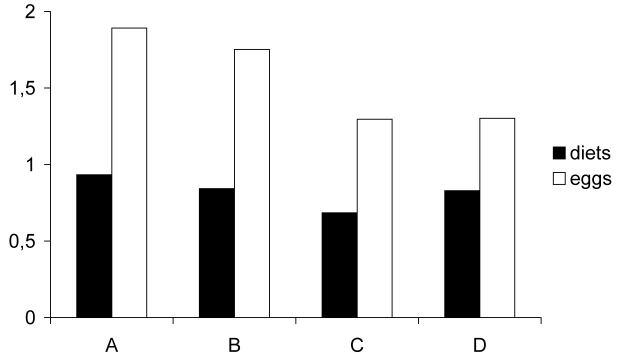

Figure 1. Polyunsaturated/monounsaturated fatty acids ratio in diets and in the eggs spawned by RB trout fed the experimental diets.

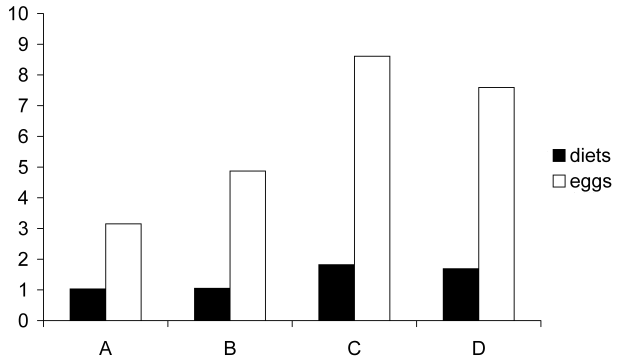

Figure 2. DHA/EPA ratio in diets and in the eggs spawned by RB trout fed the experimental diets.

eicosanoids are synthesized in minimal quantities in tissues, but they are very powerful in the control of important physiological processes in fish (Pankhurst 1998). Due to the critical importance of eicosanoids in animal physiology, it can be deduced that the n-6 series are, essential for fish species, even if at very low levels (Sargent et al. 1999). This trial indirectly confirms the ability of elongation and desaturation of C 18:2 n-6 and the preferential storage of C20:4 n-6 in rainbow trout eggs. Although not detected in the diets, AA was found in the eggs of all treatments, in spite of the long feeding period with very low levels of n-6. In the future it would be interesting to determine the rate at which lipids are used as energetic source and for plastic purpose, because then the broodstock diet could be specifically designed.

In this trial, the quality of the diet given to female rainbow trout, starting from the phase immediately preceding vitellogenesis, profoundly influenced the fatty acid composition of their eggs. Further experiments must be carried out with this plant lipid source to evaluate the effects on survival and quality of fry.

Acknowledgements

This work was supported by the Italian Ministry of University and Scientific Research (Project: Improvement of reproductive efficiency in fish).

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