JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Dietary Lipid Source Modulates in Vivo Fatty Acid Metabolism in the Freshwater Fish, Murray Cod (*Maccullochella peelii peelii*)

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The aim of the present investigation was to quantify the fate of C_{18} and long chain polyunsaturated dietary fatty acids in the freshwater fish, Murray cod, using the in vivo, whole-body fatty acid balance method. Juvenile Murray cod were fed one of five *iso*-nitrogenous, *iso*-energetic, semipurified experimental diets in which the dietary fish oil (FO) was replaced (0, 25, 50, 75, and 100%) with a blended vegetable oil (VO), specifically formulated to match the major fatty acid classes [saturated fatty acids, monounsaturated fatty acids, n-3 polyunsaturated fatty acids (PUFA), and n-6 PUFA] of cod liver oil (FO). However, the PUFA fraction of the VO was dominated by C_{18} fatty acids, while $C_{20/22}$ fatty acids were prevalent in the FO PUFA fraction. Generally, there was a clear reflection of the dietary fatty acid composition across each of the five treatments in the carcass, fillet, and liver. Lipid metabolism was affected by the modification of the dietary lipid source. The desaturation and elongation of C_{18} PUFAs increased with vegetable oil substitution, supported by the occurrence of longer and higher desaturated homologous fatty acids. However, increased elongase and desaturase activity is unlikely to fulfill the gap observed in fatty acid composition resulting from decreased highly unsaturated fatty acids intake.

KEYWORDS: ***Maccullochella peelii peelii*; whole-body fatty acid balance method; linoleic acid; α-linolenic acid; EPA; DHA**

INTRODUCTION

The steady global expansion of the aquaculture industry over the last three decades has placed a heavy dependence on capture fisheries as a source of fishmeal and fish oil (FO) for aquafeeds (*1*), two ingredients of limited supply and relatively high price. The use of these key ingredients, particularly for the culture of carnivorous fish species, has raised concerns over the sustainability of the industry and its potential to expand beyond 2010. In 2002, aquafeeds accounted for approximately 81% of the global FO production and are expected to increase to 88% by 2012 (*2*). Consequently, great emphasis has been directed toward the identification of alternative, economically sustainable feed ingredients, with plant oil sources chiefly showing the most promising results, providing that essential fatty acid (EFA) requirements are met (*3*). Many studies have demonstrated that the fatty acid composition of the fish will reflect that of the dietary lipid source (*4*, *5*). Inclusion of plant oils can therefore lead to a degradation of fatty acids associated with human health-promoting properties, in particular $C_{20/22}$ highly unsaturated fatty acids (HUFA) (*6*).

The conversion of EFA, 18:2 n-6 (linoleic acid), and 18:3 n-3 (α-linolenic acid) to long chain HUFA via an alternating series of elongation and desaturation is an innate ability demonstrated by most freshwater fish species (*7*). The efficiency of a species' capability in producing long chain HUFA can be measured both ex vivo and in vivo, and a variety of techniques are available to assess general fatty acid metabolism (*8*). Studies employing an ex vivo approach far outnumber those in which in vivo methods are employed and thus provided the emphasis for this study. In vivo methods generally utilize a whole-body approach (*9*) and are relatively simple and more informative from a quantitative view point in comparison to ex vivo counterparts. However, to date, these methods remain relatively unexplored in fish nutrition studies.

In the present study, the fatty acid metabolism of Murray cod, *Maccullochella peelii peelii* (Mitchell), was investigated using the whole-body fatty acid balance method, first proposed by Cunnane and Anderson (*9*) and later adopted and further developed by Turchini et al. (*10*). The present study was motivated by the need to better understand if the C_{18} polyunsaturated fatty acid (PUFA) elongase and desaturase capabilities of fish can compensate for the reduction of HUFA levels in fish tissues resulting from the reduction in dietary intake.

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10.1021/jf062153x CCC: $37.00
Published on Web 01/23/2007

Table 1. Ingredient and Proximate Composition of the Experimental Diets (mg g^{-1} Dry Diet) and the Fatty Acid Composition of the Experimental Diets, Expressed in mg g $Lipid^{-1}$ as Fed[a]

	dietary treatments[b]				
	FO	VO25	VO50	VO75	VO100
dietary ingredients					
casein[c]	320	320	320	320	320
gelatin	80	80	80	80	80
dextrin	100	100	100	100	100
fish meal	100	100	100	100	100
defatted soybean meal	105	105	105	105	105
wheat flour	53	53	53	53	53
FO	170	127.5	85	42.5	-
VO	-	42.5	85	127.5	170
mineral premix	40	40	40	40	40
vitamin premix	30	30	30	30	30
Cr_2O_3	2	2	2	2	2
proximate composition (mg g^{-1})					
moisture	52.5	50.1	54.2	48.4	47.7
crude protein	498.3	502.5	504.1	499.3	504.5
crude lipid	192.7	187.4	186.7	191.0	189.5
ash	48.3	48.8	48.4	48.9	49.3
NFE	208.2	211.2	206.5	212.4	209
energy (kJ/g)	19.4	19.3	19.3	19.3	19.4
fatty acid composition (mg g $lipid^{-1}$)					
14:0	43.1	35.2	23.5	15.4	6.6
16:0	135.9	154.1	159.2	181.6	206.5
16:1 n-7	62.2	47.8	31.4	18.4	4.1
18:0	34.2	36.2	32.6	34.2	35.7
18:1 n-9	152.7	188.3	207.6	250.1	295.0
18:1 n-7	30.2	27.4	20.9	18.4	14.5
18:2 n-6	41.9	59.0	69.6	88.8	109.1
18:3 n-3	8.0	64.2	115.0	176.8	242.9
18:4 n-3	11.5	9.2	6.3	3.7	1.5
20:1[d]	16.73	12.8	8.9	6.0	2.2
20:4 n-6	6.1	4.9	3.4	2.0	-
20:3 n-3	3.2	3.0	2.6	2.9	3.0
20:4 n-3	10.1	7.8	4.8	2.6	0.2
20:5 n-3	73.6	56.0	36.4	21.4	4.7
22:5 n-3	33.2	24.9	16.0	8.7	1.0
22:6 n-3	105.7	79.8	51.9	29.8	6.3
SFA	226.3	236.1	223.7	239.1	256.9
MUFA	281.3	293.1	280.4	300.1	317.8
PUFA	319.6	331.0	320.2	344.8	369.7
HUFA	235.2	179.0	117.2	68.5	15.2
n-3	245.2	244.9	232.9	245.8	259.6
n-6	59.4	73.2	79.7	94.3	109.5
n-3/n-6	4.1	3.4	2.9	2.61	2.4
HUFA/PUFA	0.7	0.5	0.4	0.20	0.0

[a] Not all fatty acids are reported. [b] Diet abbreviations: FO, 100% FO; VO25, 25% VO and 75% FO; VO50, 50% VO and 50% FO; VO75, 75% VO and 25% FO; and VO100, 100% VO. [c] Ingredient information as reported in ref *4*. [d] Represents the sum of all 20:1 isomers.

MATERIALS AND METHODS

Animals, Husbandry, and Experimental Diets. Murray cod [*M. peelii peelii* (Mitchell); Order Perciformes; Family Percichthyidae] of the 2004 year class (initial mean weight, 14 g) were obtained from Uarah fish hatchery (Grong−Grong, N.S.W., Australia) and used in this experiment. Fish were housed in a 15 tank (160 L capacity) recirculating system with an in-line oxygen generator, a physical and biological filtration plant, and an ozone disinfection unit. The system was maintained on a 12:12 h light:dark cycle at 24.7 ± 1.2 °C with a flow rate of 6 L min^{-1}. Water quality parameters were measured daily using Aquamerck test kits (Merck, Darmstadt, Germany) and maintained at optimal levels, as reported previously (*4*).

Five *iso*-nitrogenous, *iso*-energetic, *iso*-lipidic semipurified experimental diets (with minimal fish meal inclusion) were formulated with 17% lipid originating from FO (cod liver oil) and a blended vegetable oil (VO). The diets differed only with respect to the VO content, which was substituted in 25% increments (**Table 1**). The VO was formulated using three commonly available vegetable oils in the form of olive oil (12%), palm oil (43%), and linseed oil (45%) and was specifically formulated to match the major fatty acid classes of the FO. The nutritional contents of the diets were based on previous findings (*11*) and prepared and stored as also reported previously (*4*).

At the commencement of the experiment, a sample of 20 fish was taken and euthanized in excess anesthetic (0.5 mL L^{-1} benzocaine) for analysis. Four hundred fifty individually weighed (to the nearest 0.01 g) and measured (to the nearest mm) juvenile Murray cod were randomly distributed among the 15 experimental tanks (30 fish per tank) and assigned one of the five dietary treatments (three replicates per treatment). Fish were fed twice daily at approximately 08:00 and 15:00 h to apparent satiation for a period of 98 days. At the termination of the experiment, a random sample of 24 fish (eight per tank) from each treatment were euthanized for analyses. Fecal samples were collected on a daily basis. Prior to collection, tanks were siphoned to remove uneaten feed from the pm feed and avoid possible contamination. The following morning, feces were removed from each tank, freeze-dried, and stored at −20 °C at until analyzed.

Chemical Analyses. Sampled fish were randomly split into two groups of four fish for whole-body fatty acid balance analysis/proximate analysis and tissue fatty acid analysis. Proximate analysis was conducted using standard procedures (*12*). The percentage moisture was determined by drying in an oven at 80 °C until a constant weight was achieved; protein (Kjeldahl nitrogen; N × 6.25) was determined in an automated Kjeltech (model 2300, Tecator, Sweden); total lipid was determined by chloroform/methanol extraction (2:1 v/v) (*13*); ash was determined by incineration in a muffle furnace (model WIT, C & L Tetlow, Australia) at 550 °C for 18 h; and gross energy was determined using a ballistic bomb calorimeter (Gallenkamp, United Kingdom).

Fatty acid analysis was performed in triplicate on each of the experimental diets and fecal samples. Tissue samples (fillet, liver, and whole-body) were pooled (four fish) and analyzed in triplicate per replicate. Following lipid extraction, fatty acids were esterified into methyl esters using the acid-catalyzed methylation method (*14*) and followed the methods previously used in the laboratory (*15*, *16*). Briefly, 250 μL of ethyl 13:0 (5 mg/mL^{-1}) (Sigma-Aldrich, Inc., St. Louis, MO) was added to monitor the extent of transesterification, and 800 μL of 23:0 (2.5 mg/mL^{-1}) was used as an internal standard (Sigma-Aldrich, Inc.). Fatty acid methyl esters were isolated and identified using a Shimadzu GC 17A (Shimadzu, Chiyoda-ku, Tokyo, Japan) equipped with an Omegawax 250 capillary column (30 m × 0.25 mm internal diameter, 25 μm film thickness, Supelco, Bellefonte, PA), a flame ionization detector (FID), a Shimadzu AOC-20i autoinjector, and a split injection system (split ratio, 50:1). The temperature program was 150−180 °C at 3 °C min^{-1}, then from 180 to 250 °C at 2.5 °C min^{-1}, and held at 250 °C for 10 min. The carrier gas was helium at 1.0 mL min^{-1} at a constant flow. Each of the fatty acids was identified relative to known external standards. The resulting peak areas were then corrected by theoretical relative FID response factors (*17*) and quantified relative to the internal standard. Feed and freeze-dried fecal samples were analyzed for proximate and fatty acid analysis and chromic oxide (Cr_2O_3) according to the method of Furukawa and Tsukahara (*18*). Estimates of lipid digestibility (%LD) and fatty acid digestibility were calculated using standard formulas (*19*).

Calculations. The computation of the whole-body fatty acid balance was carried out as previously described by Turchini et al. (*10*). In detail, the individual concentrations of fatty acids in the diets, feces, and the initial and final carcass were expressed in mg per fish. It was then possible to calculate the appearance or disappearance of individual fatty acids (FA) according to the following formulas:

$$\text{FA intake} = \text{g of feed intake} \times \text{mg of FA per g of feed}$$

$$\text{FA excretion} = \text{mg of FA intake} \times \text{FA digestibility}$$

$$\text{FA accumulation} = \text{mg of FA in final carcass} - \text{mg of FA in initial carcass}$$

$$\text{FA appearance/disappearance} = \text{FA accumulation} - \text{FA intake} - \text{FA excretion}$$

The disappearance of fatty acids can be attributed to their elongation and desaturation to longer chain fatty acids or utilization of their carbon skeleton via β-oxidation for energy production. In order to determine

Table 2. Main Growth, Biometry and Whole-Body, Fillet, and Liver Proximate Composition (in mg g^{-1}) Wet Weight of Juvenile Murray Cod Fed Different Experimental Diets over a 98 Day Period[a]

	dietary treatments[b]						
	initial[c]	FO	VO25	VO50	VO75	VO100	R^2
				growth parameters			
initial weight		14.3 ± 0.2	14.5 ± 0.2	14.5 ± 0.2	14.4 ± 0.1	14.6 ± 0.4	0.58
final weight		62.3 ± 3.4 b	58.9 ± 0.5 ab	58.9 ± 1.5 ab	52.8 ± 2.4 ab	49.9 ± 2.2 a	0.93**
SGR[d]		1.50 ± 0.1 b	1.43 ± 0.1 ab	1.43 ± 0.1 ab	1.32 ± 0.1 ab	1.25 ± 0.1 a	0.93**
FCR[e]		0.77 ± 0.1	0.78 ± 0.1	0.75 ± 0.1	0.80 ± 0.1	0.83 ± 0.1	0.50
FDR[f]		1.85 ± 0.1	1.78 ± 0.1	1.70 ± 0.1	1.64 ± 0.1	1.58 ± 0.1	0.99***
HSI%[g]		1.93 ± 0.2	1.77 ± 0.1	2.06 ± 0.1	1.89 ± 0.1	2.17 ± 0.2	0.38
VFI%[h]		3.69 ± 0.5	2.97 ± 0.2	2.88 ± 0.3	2.69 ± 0.2	2.90 ± 0.1	0.59
				whole-body			
moisture	744.4 ± 1.7	711.4 ± 1.9	711.5 ± 1.0	720.9 ± 2.5	717.6 ± 4.1	710.1 ± 1.4	0.01
protein	150.7 ± 1.2	152.5 ± 7.1	161.8 ± 3.4	152.8 ± 2.9	156.9 ± 1.2	157.7 ± 3.7	0.05
lipid	76.2 ± 2.6	108.5 ± 2.6	108.8 ± 7.9	100.2 ± 2.8	104.7 ± 4.8	106.2 ± 3.3	0.16
ash	35.6 ± 0.4	30.9 ± 2.4	32.7 ± 0.5	30.4 ± 0.6	30.8 ± 0.8	34.6 ± 1.2	0.25
				fillet			
moisture	788.9 ± 0.8	782.7 ± 1.9	775.2 ± 1.4	781.8 ± 3.1	777.0 ± 1.1	781.9 ± 1.1	0.00
protein	192.4 ± 0.1	184.8 ± 3.3	191.0 ± 3.5	184.7 ± 1.9	194.0 ± 0.8	187.6 ± 1.9	0.12
lipid	17.2 ± 0.5	27.0 ± 1.4	29.4 ± 0.6	30.9 ± 2.1	26.1 ± 1.6	30.1 ± 1.6	0.05
ash	11.6 ± 0.2	11.0 ± 0.4	10.6 ± 0.3	10.9 ± 0.2	11.1 ± 0.1	10.8 ± 0.5	0.00
				liver			
moisture	781.7 ± 4.0	715.4 ± 8.7 b	715.1 ± 6.1 b	689.2 ± 5.9 b	688.4 ± 13.8 b	636.3 ± 9.2 a	0.82*
protein	111.6 ± 0.4	106.9 ± 4.7	96.0 ± 1.6	91.7 ± 5.1	104.9 ± 11.7	85.3 ± 3.5	0.36
lipid	83.4 ± 3.9	108.2 ± 3.7 a	105.7 ± 6.1 a	110.4 ± 6.4 a	148.3 ± 12.3 a	205.7 ± 16.4 b	0.77
ash	14.8 ± 0.1	10.9 ± 0.5 ab	11.4 ± 0.6 a	10.3 ± 0.3 ab	10.8 ± 0.3 ab	9.4 ± 0.2 b	0.55

[a] Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA. Data were also analyzed with the linear regression relative to the degree of substitution of FO with the blended vegetable oil; the coefficient of determination R^2 and P values (*, <0.05; **, <0.01; and ***, <0.001) are reported. [b] See **Table 1** for diet abbreviations. [c] Statistical analyses not performed on initial sample. [d] SGR: SGR (% day^{-1}) = [Ln(final weight) − Ln(initial weight)] × (number of days)$^{-1}$ × 100. [e] FCR: FCR = (dry feed fed) × (wet weight gain)$^{-1}$. [f] FDR: FDR = [Ln(final lipid) − Ln(initial lipid)] × (number of days)$^{-1}$. [g] HSI%: HSI% = (weight of liver) × (total fish weight)$^{-1}$ × 100. [h] VFI%: VFI% = (visceral fat weight) × (total fish weight)$^{-1}$ × 100.

the partitioning of fatty acids in the computation of the 18:2 n-6 and 18:3 n-3 balance, the appearance and disappearance of fatty acids were converted to mmol per fish and the number of mmol of longer chain fatty acids that had appeared was subtracted from the number of mmol of the relative previous fatty acid in the fatty acid elongation/desaturation pathway. For example, the mathematical model used for the whole-body fatty acid balance computation of n-6 fatty acids can be described by the following equations [where: ϵ = total specified FA converted (elongated or desaturated); δ = number of mmol of the specified FA appeared/disappeared; if δ is a negative number (FA disappearance = oxidized), then $\delta = 0$ for the following computation]:

$$\epsilon(20{:}4\ \text{n-6}) = \delta(22{:}4\ \text{n-6})$$

$$\epsilon(20{:}3\ \text{n-6}) = \delta(20{:}4\ \text{n-6}) + \epsilon(20{:}4\ \text{n-6})$$

$$\epsilon(18{:}3\ \text{n-6}) = \delta(20{:}3\ \text{n-6}) + \epsilon(20{:}3\ \text{n-6})$$

$$\epsilon(20{:}2\ \text{n-6}) = \delta(22{:}2\ \text{n-6})$$

$$\epsilon(18{:}2\ \text{n-6}) = \delta(18{:}3\ \text{n-6}) + \epsilon(18{:}3\ \text{n-6}) + \delta(20{:}2\ \text{n-6}) + \epsilon(20{:}2\ \text{n-6})$$

The total 18:2 n-6 balance is then delineated by the following equations [where: DE(18:2 n-6) = the total amount of 18:2 n-6 elongated to dead end products; NP(18:2 n-6) = the total amount of 18:2 n-6 converted through the normal elongation/desaturation pathway; Ox(18:2 n-6) = the total amount of 18:2 n-6 oxidized; and γ(18:2 n-6) = total number of mmol of 18:2 n-6 disappeared]:

$$\text{DE}(18{:}2\ \text{n-6}) = \delta(20{:}2\ \text{n-6}) + \epsilon(20{:}2\ \text{n-6})$$

$$\text{NP}(18{:}2\ \text{n-6}) = \delta(18{:}3\ \text{n-6}) + \epsilon(18{:}3\ \text{n-6})$$

$$\text{Ox}(18{:}2\ \text{n-6}) = \gamma(18{:}2\ \text{n-6}) - \text{DE(LA)} - \text{NP(LA)}$$

Ultimately, it is possible to estimate the elongase, Δ-5, and Δ-6 desaturase activities, expressed as mmol of product per gram of fish (average fish weight) per day, with the following equations:

$$\text{g of fish day}^{-1} = (\text{initial fish weight} + \text{final fish weight}) \times 2^{-1} \times \text{no. of days}^{-1}$$

$$\Delta\text{-6 desaturase} = [\delta(18{:}3\ \text{n-6}) + \epsilon(18{:}3\ \text{n-6})]\ (\text{g of fish day}^{-1})^{-1}$$

$$\Delta\text{-5 desaturase} = [\delta(20{:}4\ \text{n-6}) + \epsilon(20{:}4\ \text{n-6})]\ (\text{g of fish day}^{-1})^{-1}$$

$$\text{elongase} = [\delta(20{:}2\ \text{n-6}) + \epsilon(20{:}2\ \text{n-6}) + \epsilon(18{:}3\ \text{n-6}) + \epsilon(20{:}4\ \text{n-6})]\ (\text{g of fish day}^{-1})^{-1}$$

Statistical Analysis. All data were reported as means ± standard error ($n = 3$). Data interpretation was based on two different statistical tests: Data were analyzed among treatments by (i) linear regression (relative to the degree of FO substitution at significant levels of 0.05, 0.01, and 0.001%) and (ii) one-way analysis of variance (ANOVA) at a significance level of 0.05 following confirmation of normality and homogeneity of variance. Where significant differences were detected by ANOVA, data were subjected to a Student−Newman−Keuls posthoc test for homogeneous subsets. All statistical analyses were computed using SPSS v12.0.1 (SPSS Inc., Chicago, IL).

RESULTS

The test diets were *iso*-nitrogenous, *iso*-lipidic, and *iso*-energetic, and their fatty acid composition reflected that of the

Table 3. Fillet Fatty Acid Composition of Juvenile Murray Cod Fed Different Experimental Diets over a 98 Day Period, Expressed in mg g Lipid^{-1}[a]

	dietary treatments[b]						
	initial[c]	FO	VO25	VO50	VO75	VO100	R^2
14:0	22.5 ± 0.4	28.3 ± 1.3 d	19.5 ± 3.9 bc	16.0 ± 2.9 abc	12.8 ± 0.4 ab	9.6 ± 0.4 a	0.94**
16:0	125.8 ± 2.1	141.5 ± 1.2 a	142.2 ± 4.5 a	156.4 ± 5.9 c	148.3 ± 1.7 bc	160.5 ± 3.1 c	0.68
16:1 n-7	30.3 ± 0.8	48.8 ± 1.6 e	39.9 ± 2.0 d	35.0 ± 2.2 c	21.9 ± 0.6 b	15.7 ± 0.6 a	0.98***
18:0	44.7 ± 0.6	35.9 ± 0.2	33.9 ± 0.5	35.1 ± 1.2	34.7 ± 0.5	36.5 ± 0.9	0.11
18:1 n-9	118.8 ± 1.9	131.7 ± 4.1 a	153.3 ± 3.3 b	184.1 ± 9.8 c	187.6 ± 3.1 c	225.1 ± 6.6 d	0.96**
18:1 n-7	23.6 ± 0.5	27.0 ± 0.8 d	24.4 ± 0.2 c	22.1 ± 1.1 c	17.7 ± 0.1 b	14.6 ± 0.6 a	0.99***
18:2 n-6	35.8 ± 0.7	31.8 ± 1.7 a	44.0 ± 0.9 b	56.5 ± 2.8 c	63.7 ± 1.0 d	79.6 ± 1.1 e	0.99***
18:3 n-3	7.1 ± 0.3	5.5 ± 0.3 a	36.2 ± 1.3 b	70.9 ± 4.6 c	85.9 ± 1.9 d	113.3 ± 3.1 e	0.99***
18:4 n-3	7.3 ± 0.8	6.5 ± 0.4 a	6.7 ± 0.6 a	8.0 ± 0.4 a	8.8 ± 0.5 a	16.5 ± 0.4 b	0.71
20:1[d]	7.2 ± 1.1	10.7 ± 0.8 d	9.2 ± 0.4 cd	7.9 ± 0.2 c	5.5 ± 0.5 ab	4.5 ± 0.3 a	0.98***
20:4 n-6	4.9 ± 4.9	8.4 ± 0.3	6.9 ± 0.2	5.5 ± 0.4	5.0 ± 0.1	2.5 ± 0.4	0.96**
20:3 n-3	15.3 ± 1.5	7.6 ± 1.6 a	7.3 ± 1.1 a	8.5 ± 1.5 a	11.1 ± 0.4 ab	10.9 ± 0.9 ab	0.83*
20:4 n-3	6.2 ± 1.0	8.0 ± 0.9 a	6.9 ± 0.2 a	7.2 ± 0.2 a	7.9 ± 0.1 a	13.1 ± 0.2 b	0.49
20:5 n-3	43.9 ± 1.1	37.9 ± 0.9 e	29.0 ± 0.9 d	20.9 ± 0.4 c	14.5 ± 0.5 b	9.6 ± 0.6 a	0.99***
22:5 n-3	29.5 ± 0.4	35.6 ± 1.3 e	29.3 ± 0.8 d	23.9 ± 0.7 c	17.1 ± 0.3 b	13.8 ± 0.3 a	0.99***
22:6 n-3	136.2 ± 2.1	121.9 ± 2.2 e	99.4 ± 2.1 d	81.8 ± 0.7 c	68.8 ± 2.4 b	38.9 ± 2.1 a	0.98***
SFA	212.6 ± 3.7	213.3 ± 3.7	203.6 ± 8.4	213.6 ± 8.1	199.4 ± 3.1	211.5 ± 2.2	0.04
MUFA	188.7 ± 2.5	228.7 ± 6.2 a	234.9 ± 5.9 ab	255.6 ± 13.0 ab	237.4 ± 4.5 ab	262.8 ± 7.1 b	0.59
PUFA	305.2 ± 9.1	282.5 ± 6.4	283.4 ± 7.0	298.1 ± 8.6	293.7 ± 4.2	312.7 ± 2.8	0.82*
HUFA	240.0 ± 4.7	222.7 ± 4.0 e	181.8 ± 3.4 d	150.7 ± 1.6 c	126.9 ± 2.5 b	91.8 ± 4.5 a	0.99***
n-3	245.5 ± 2.8	223.0 ± 4.2	214.8 ± 5.7	221.2 ± 5.5	214.1 ± 2.2	216.2 ± 2.0	0.33
n-6	50.7 ± 6.1	50.2 ± 2.6 a	60.4 ± 0.2 a	70.4 ± 2.5 b	76.8 ± 1.3 b	93.8 ± 0.6 c	0.98**
n-3/n-6	5.0 ± 0.7	4.5 ± 0.2 c	3.6 ± 0.1 b	3.1 ± 0.1 ab	2.8 ± 0.1 ab	2.3 ± 0.1 a	0.96**
HUFA/PUFA	0.8 ± 0.0	0.8 ± 0.0 e	0.6 ± 0.0 d	0.5 ± 0.0 c	0.4 ± 0.0 b	0.3 ± 0.0 a	0.99***

[a] Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA. Not all fatty acids are reported. Data were also analyzed with linear regression relative to the degree of substitution of FO with the blended vegetable oil; the coefficient of determination R^2 and P values (*, <0.05; **, <0.01; and ***, <0.001) are reported. [b] See **Table 1** for diet abbreviations. [c] Statistical analyses not performed on initial sample. [d] Represents the sum of all 20:1 isomers.

added oils (**Table 1**). As the VO was formulated to mimic the major class profile of cod liver oil (FO), the diets were similar in this respect [i.e., saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), PUFA, n-3, and n-6]. However, there were differences in the fatty acid profiles with respect to HUFA and C_{18}PUFA classes and individual fatty acids. The FO diet was characterized by high levels of 20:5 n-3 and 22:6 n-3, while the VO100 diet was high in 16:0, 18:2 n-6, 18:3 n-3, and in particular 18:1 n-9.

Juvenile Murray cod tripled their initial weight over the experimental period. Significant differences ($P < 0.05$) were observed between fish receiving the FO and VO100 diet in mean final weight, with values of 62.3 and 49.9 g, respectively (**Table 2**). Similarly, specific growth rate (% day^{-1}) (SGR) and fat deposition rate (FDR) exhibited the same trend, ranging from 1.50 to 1.25 and 1.85 to 1.58, respectively. No significant differences were apparent between treatments for the feed conversion ratio (FCR), hepatosomatic index (HSI%), or visceral fat index (VFI%). The lipid content of the liver was significantly modified by the inclusion of 100% VO, with values around 2-fold of the FO, VO25, and VO50 treatments. The proximate composition of the whole-body and fillet did not differ significantly between the dietary treatments.

The fillet fatty acid compositions of fish reared on the experimental diets are given in **Table 3**. Increased VO substitution resulted in a significant ($P < 0.05$) amplification of 18:1 n-9, 18:2 n-6, and 18:3 n-3 in Murray cod fillet. Adversely, increasing VO substitution levels had a significant impact on levels of n-3 and n-6 HUFA, particularly, 20:5 n-3, 22:6 n-3, and 20:4 n-6. The fatty acid composition of Murray cod liver exhibited a similar trend, although levels of MUFA were found in higher concentrations than levels in the diet (**Table 4**).

Individual differences in the fatty acid composition of the experimental diets resulted in a significantly lower intake, excretion, final body content, and accumulation of n-3 and n-6 HUFA as the substitution of FO approached 100% (**Table 5**). No difference was noted in the intake or final body content of SFA, MUFA, and n-3 PUFA. However, the excretion of MUFA, n-3 PUFA, and n-6 PUFA increased as the level of VO in the diet increased. An appearance of 31.5 mg of n-3 HUFA per fish and 5.4 mg of n-6 HUFA per fish in Murray cod receiving the VO100 diet was recorded.

With respect to the n-6 fatty acid balance, there was an accumulation and significant appearance of 18:3 n-6 and 20:3 n-6 in the VO100 treatment despite a low to nonexistent intake in the diet (**Table 6**). Conversely, with regard to the total balance of 18:2 n-6, a significantly higher ($P < 0.05$) disappearance was recorded in Murray cod receiving the VO100 treatment. The disappearance of 20:4 n-6 was positively correlated to the level of VO substitution ($R^2 = 0.96$, $P < 0.01$). However, 20:4 n-6 was not present in the intake of fish receiving the VO100 treatment and recorded a marginal disappearance (-1.4 ± 0.1 mg per fish).

The n-3 fatty acid balance is given in **Table 7**. The total intake and disappearance of 18:3 n-3 were positively correlated with the inclusion of VO in the diet ($R^2 > 0.99$, $P < 0.001$ and $R^2 = 0.97$, $P < 0.01$, respectively). Fatty acids on the n-3 elongation/desaturation pathway, namely, 18:4 n-3 and 20:4 n-3 in the VO50, VO75, and VO100 treatments, recorded an appearance ($P < 0.05$). The intake of 20:5 n-3 and 22:6 n-3 was negatively correlated with the inclusion of VO ($R^2 = 0.99$, $P < 0.001$ and $R^2 = 0.98$, $P < 0.001$, respectively). However, the 20:5 n-3 and 22:6 n-3 balance, although negative across all treatments, approached appearance as the level of VO substitution in the diets advanced to 100% (-39.4 ± 2.7 and -13.3 ± 4.5 mg per fish, respectively).

The majority of the net intake of 18:1 n-9, 18:2 n-6, and 18:3 n-3 was accumulated, ranging from 73.6 to 80.4% in 18:1 n-9,

Table 4. Liver Fatty Acid Composition of Juvenile Murray Cod Fed Different Experimental Diets over a 98 Day Period, Expressed in mg g Lipid^{-1}[a]

	dietary treatments[b]						
	initial[c]	FO	VO25	VO50	VO75	VO100	R^2
14:0	14.9 ± 1.2	5.1 ± 4.4	12.9 ± 2.3	11.9 ± 2.2	12.4 ± 0.2	11.0 ± 0.5	0.32
16:0	98.9 ± 5.4	152.4 ± 0.2 a	168.0 ± 7.7 a	202.4 ± 7.8 b	206.2 ± 8.6 b	222.3 ± 9.2 b	0.94**
16:1 n-7	23.9 ± 3.2	66.2 ± 0.4 ab	60.8 ± 4.5 ab	70.9 ± 2.2 b	62.4 ± 4.2 ab	54.1 ± 2.8 a	0.33
18:0	34.6 ± 0.3	30.7 ± 0.4 a	34.4 ± 0.3 ab	40.0 ± 2.2 bc	38.8 ± 2.3 bc	46.0 ± 2.4 c	0.91*
18:1 n-9	100.8 ± 12.7	186.1 ± 0.1	204.5 ± 6.1	258.8 ± 5.7	295.2 ± 9.1	296.5 ± 61.2	0.93**
18:1 n-7	22.3 ± 1.9	36.3 ± 0.8	32.2 ± 0.9	33.8 ± 0.4	28.3 ± 1.5	79.1 ± 53.3	0.38
18:2 n-6	27.3 ± 2.4	27.1 ± 0.3 a	31.9 ± 3.1 ab	37.9 ± 1.4 b	49.6 ± 2.4 c	58.7 ± 1.7 d	0.97**
18:3 n-3	5.2 ± 0.7	3.6 ± 0.3 a	19.7 ± 1.7 b	32.4 ± 1.7 c	47.4 ± 2.3 d	55.2 ± 2.7 e	0.99***
18:4 n-3	2.9 ± 1.6	4.0 ± 0.6 a	4.5 ± 0.2 a	6.2 ± 0.2 b	10.1 ± 0.1 c	16.9 ± 0.2 d	0.86*
20:1[d]	8.9 ± 1.2	11.6 ± 0.1 b	9.6 ± 1.1 a	9.0 ± 0.4 a	8.8 ± 0.2 a	7.7 ± 0.1 a	0.89*
20:4 n-6	7.7 ± 3.9	6.9 ± 0.3 e	5.6 ± 0.2 d	4.6 ± 0.5 c	2.2 ± 0.3 b	0.5 ± 0.1 a	0.98**
20:3 n-3	18.7 ± 2.7	4.0 ± 0.3	6.1 ± 0.8	7.2 ± 0.7	6.7 ± 0.3	5.5 ± 0.4	0.22
20:4 n-3	6.4 ± 0.4	5.9 ± 0.1 a	6.2 ± 0.7 a	7.3 ± 0.3 a	10.6 ± 0.9 b	13.0 ± 0.9 c	0.91*
20:5 n-3	23.2 ± 1.9	17.4 ± 0.1 e	13.1 ± 0.7 d	8.6 ± 0.2 c	5.6 ± 0.3 b	2.7 ± 0.1 a	0.99***
22:5 n-3	35.0 ± 1.3	33.8 ± 1.7 d	23.9 ± 2.4 c	14.9 ± 1.1 b	12.1 ± 1.2 ab	7.3 ± 0.5 a	0.95**
22:6 n-3	156.9 ± 7.6	136.2 ± 6.5 e	99.4 ± 8.4 d	62.3 ± 2.6 c	40.7 ± 3.7 b	13.5 ± 0.5 a	0.99***
SFA	159.2 ± 4.0	195.9 ± 3.3 a	222.0 ± 6.9 a	259.7 ± 9.4 b	262.6 ± 10.1 b	283.8 ± 11.9 b	0.94**
MUFA	170.0 ± 17.7	309.0 ± 1.3 a	314.1 ± 5.6 a	378.7 ± 7.4 b	399.5 ± 12.5 b	439.9 ± 14.1 c	0.95**
PUFA	299.7 ± 13.2	256.7 ± 7.5 b	225.4 ± 18.5 ab	197.6 ± 5.2 a	199.6 ± 12.3 a	190.9 ± 7.0 a	0.84*
HUFA	249.4 ± 6.7	208.9 ± 8.1 e	158.3 ± 13.3 d	108.7 ± 4.4 c	80.9 ± 6.9 b	45.2 ± 2.1 a	0.99***
n-3	248.3 ± 8.0	205.0 ± 6.9 c	172.8 ± 14.4 b	139.0 ± 4.7 a	133.1 ± 8.6 a	114.0 ± 4.9 a	0.94**
n-6	45.7 ± 3.8	45.8 ± 0.7 a	47.5 ± 3.7 a	54.6 ± 1.2 a	64.2 ± 3.2 b	75.5 ± 1.8 c	0.94**
n-3/n-6	5.5 ± 0.3	4.5 ± 0.1 e	3.6 ± 0.1 d	2.6 ± 0.1 c	2.1 ± 0.1 b	1.5 ± 0.1 a	0.98**
HUFA/PUFA	0.8 ± 0.0	0.8 ± 0.0 e	0.7 ± 0.0 d	0.6 ± 0.0 c	0.4 ± 0.0 b	0.2 ± 0.0 a	0.99***

[a] Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA. Not all fatty acids are reported. Data were also analyzed with linear regression relative to the degree of substitution of FO with the blended vegetable oil; the coefficient of determination R^2 and P values (*, <0.05; **, <0.01; and ***, <0.001) are reported. [b] See **Table 1** for diet abbreviations. [c] Statistical analyses not performed on initial sample. [d] Represents the sum of all 20:1 isomers.

from 61.2 to 69.9% in 18:2 n-6, and from 47.5 to 62.6% in 18:3 n-3 (**Figure 1**). Consequently, a lower percentage of 18:1 n-9, 18:2 n-6, and 18:3 n-3 was oxidized (19.6−26.4, 29.1−34.6, and 36.9−42.3%, respectively).

Following the direction of the normal EFA elongation/desaturation pathway, significantly higher amounts ($P < 0.05$) of 18:2 n-6 and 18:3 n-3 in fish receiving the VO100 were elongated and desaturated. Total elongase and Δ-6 desaturase activity of 18:2 n-6 is shown in **Figure 2**. Elongase and Δ-6 desaturase activity increased with VO substitution. Fish receiving the VO100 treatment had significantly higher ($P < 0.05$) elongase and Δ-6 desaturase activity as compared to those of the other treatments (0.009 ± 0.0003 and 0.035 ± 0.007 mmol/g of fish/day, respectively). In fish receiving the VO75 and VO100 treatments, the Δ-6 desaturase activity was significantly higher than elongase activity. Similarly, elongase and Δ-6 desaturase activity in 18:3 n-3 increased with VO substitution ($P < 0.05$) and was greatest in fish receiving the VO100 treatment (0.09 ± 0.005 and 0.15 ± 0.006 mmol/g of fish/day, respectively) (**Figure 3**). The Δ-6 desaturase activity was significantly higher than the elongase activity in fish receiving the VO75 and VO100 treatments.

A direct elongation of 18:2 n-6 and 18:3 n-3 toward dead end products was recorded, and fatty acid dead end products derived from 18:3 n-3 (i.e., 20:3 n-3) in the VO100 treatment were greater ($P < 0.05$) than those derived from 18:2 n-6 (i.e., 20:2 n-6 and 22:2 n-6) (data not reported).

DISCUSSION

The inclusion of a blended vegetable oil alternative to FO in a semipurified diet for Murray cod had no effect on mortality, palatability, or feed efficiency. However, a reduction in growth performance was evident with the increasing inclusion level. This result is consistent with a previous study in which Murray cod were fed various alternative vegetable oil lipid sources in a semipurified diet (*4*) and studies of other warm water freshwater fish species in which the FO component was substituted in a similar manner (*20*, *21*). Contrary to this, studies on species including Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), reported no significant growth effects resulting from vegetable oil substitution (*22*, *23*). A possible explanation for these differences in growth may be related to the amount of fishmeal in the experimental diets and the capability of species such as Atlantic salmon to effectively utilize dietary lipid to spare protein from energy production. On the other hand, species such as Murray cod are unable to effectively utilize the protein sparing capability and are not so efficient in utilizing lipid for energy production, and the modification in quality of the dietary lipid source may be responsible for growth reduction.

In the present study, the lipid content of the fillet ranged from 26.1 ± 1.6 to 30.9 ± 2.1 mg g^{-1} and did not differ between dietary treatments. However, there was a significant difference ($P < 0.05$) in the amount of lipid found within Murray cod livers of fish receiving the VO100 treatment. In previous Murray cod studies in which FO was substituted with linseed or canola oil, the liver lipid concentration increased as the dietary level of the substitute oil reached 100% (*4*). This is consistent with results obtained by Tocher et al. (*24*) for Atlantic salmon receiving diets substituted with linseed oil where increased liver lipid levels were attributable to increased levels of neutral lipids.

The fatty acid composition of fish tissue, as demonstrated previously for Murray cod (*4*) and as is well-documented for other fish species (*21*, *22*), closely reflected that of the dietary lipid source. This resulted in a loss of the human health-promoting factors associated, in particular, with n-3 HUFA.

A variety of methods have been developed to estimate elongase and desaturase activity in animal studies. Of these, the most widely adopted method for fish nutritional studies is an ex vivo assessment method in which tissue microsomes or

Table 5. Fatty Acid Class Balance of Juvenile Murray Cod Fed Different Experimental Diets over a 98 Day Period; Data Represent the Total Amount of Fatty Acid (mg) Per Fish[a]

	dietary treatments[b]					
	FO	VO25	VO50	VO75	VO100	R^2
			SFA			
intake	1774.6 ± 51.9	1666.5 ± 59.6	1504.7 ± 88.5	1531.0 ± 97.5	1568.6 ± 82.3	0.61
excretion	73.8 ± 5.8	79.7 ± 5.6	83.4 ± 12.4	125.8 ± 34.1	130.0 ± 30.5	0.86*
initial body content	252.1 ± 2.7	254.4 ± 3.7	254.5 ± 4.0	253.4 ± 2.0	256.7 ± 6.5	0.58
final body content	1544.8 ± 100.0	1347.9 ± 56.4	1318.2 ± 84.1	1243.0 ± 105.4	1173.1 ± 88.8	0.91*
accumulation	1292.7 ± 100.9	1093.4 ± 52.7	1063.7 ± 87.6	989.6 ± 107.3	916.5 ± 85.0	0.91*
appearance/disappearance	–408.1 ± 58.9	–493.3 ± 18.6	–357.6 ± 65.8	–415.6 ± 40.4	–522.1 ± 78.2	0.13
			MUFA			
intake	2205.8 ± 64.5	2068.7 ± 74.0	1886.2 ± 110.9	1921.9 ± 122.5	1940.5 ± 101.8	0.66
excretion	97.9 ± 9.9 a	116.2 ± 5.3 a	106.5 ± 11.6 a	154.2 ± 9.3 b	148.5 ± 8.1 b	0.76
initial body content	308.9 ± 3.4	311.7 ± 4.6	311.8 ± 4.9	310.5 ± 2.5	314.5 ± 8.0	0.58
final body content	1910.5 ± 125.2	1769.2 ± 88.1	1744.2 ± 107.6	1752.7 ± 144.0	1692.0 ± 107.8	0.77
accumulation	1601.6 ± 126.6	1457.5 ± 83.6	1432.5 ± 111.7	1442.3 ± 146.3	1377.5 ± 104.4	0.77
appearance/disappearance	–506.4 ± 80.5	–495.0 ± 31.0	–347.2 ± 83.2	–325.5 ± 35.7	–414.5 ± 81.0	0.46
			n-3 PUFA			
intake	1922.9 ± 56.2	1728.5 ± 61.8	1566.9 ± 92.1	1574.3 ± 100.3	1584.9 ± 83.2	0.73
excretion	43.5 ± 5.2 a	46.8 ± 1.0 a	44.2 ± 5.3 a	64.3 ± 3.7 b	67.5 ± 3.1 b	0.79*
initial body content	232.0 ± 2.5	234.1 ± 3.4	234.1 ± 3.7	233.1 ± 1.9	236.2 ± 6.0	0.58
final body content	1391.4 ± 87.8	1290.8 ± 65.1	1226.8 ± 79.4	1197.8 ± 96.9	1116.0 ± 61.0	0.97**
accumulation	1159.4 ± 89.3	1056.7 ± 62.2	992.7 ± 82.3	964.6 ± 98.5	879.9 ± 58.8	0.97**
appearance/disappearance	–720.1 ± 56.2	–625.0 ± 42.1	–530.0 ± 74.7	–545.4 ± 6.7	–637.6 ± 65.7	0.25
			n-6 PUFA			
intake	465.8 ± 13.6 a	516.8 ± 18.5 ab	536.0 ± 31.5 ab	603.7 ± 38.5 bc	668.4 ± 35.1 c	0.97**
excretion	17.8 ± 1.7 a	20.3 ± 0.6 a	19.6 ± 2.6 a	30.6 ± 1.6 b	33.4 ± 1.7 b	0.85*
initial body content	77.8 ± 0.8	78.5 ± 1.2	78.5 ± 1.2	78.2 ± 0.6	79.2 ± 2.0	0.58
final body content	374.4 ± 20.6	409.6 ± 22.3	440.6 ± 27.7	484.4 ± 41.9	500.3 ± 31.0	0.99***
accumulation	296.7 ± 21.0	331.1 ± 21.3	362.1 ± 28.7	406.2 ± 42.4	421.1 ± 30.5	0.98***
appearance/disappearance	–151.3 ± 12.1	–165.5 ± 11.9	–154.2 ± 25.0	–166.9 ± 5.1	–213.9 ± 31.9	0.63
			n-3 HUFA			
intake	1770.0 ± 51.7 e	1210.6 ± 43.3 d	750.8 ± 44.1 c	418.7 ± 26.7 b	92.6 ± 4.9 a	0.98***
excretion	39.6 ± 4.3 d	31.9 ± 0.7 c	21.6 ± 2.3 b	18.1 ± 1.1 ab	11.2 ± 1.1 a	0.98**
initial body content	209.3 ± 2.3	211.2 ± 3.1	211.3 ± 3.3	210.4 ± 1.7	213.1 ± 5.4	0.58
final body content	1286.7 ± 84.7 e	969.2 ± 49.5 d	705.3 ± 37.0 c	499.4 ± 31.7 b	326.0 ± 6.4 a	0.99***
accumulation	1077.4 ± 86.1 e	758.0 ± 47.0 d	494.1 ± 39.57 c	289.0 ± 33.1 b	112.9 ± 6.6 a	0.99***
appearance/disappearance	–653.0 ± 55.3 a	–420.7 ± 36.4 b	–235.2 ± 37.1 c	–111.5 ± 9.6 d	31.5 ± 9.2 e	0.98***
			n-6 HUFA			
intake	74.4 ± 2.2 d	53.0 ± 1.9 c	39.1 ± 2.3 b	19.8 ± 1.3 a		0.99***
excretion	2.9 ± 0.6 b	1.3 ± 0.1 a	0.6 ± 0.3 a	0.6 ± 0.1 a		0.83*
initial body content	12.7 ± 0.1	12.8 ± 0.2	12.8 ± 0.2	12.7 ± 0.1	12.9 ± 0.3	0.58
final body content	62.0 ± 3.0 e	49.0 ± 2.8 d	38.0 ± 2.4 c	28.1 ± 2.3 b	18.3 ± 1.6 a	0.99***
accumulation	49.4 ± 3.0 e	36.2 ± 2.6 d	25.2 ± 2.6 c	15.4 ± 2.4 b	5.4 ± 1.4 a	0.99***
appearance/disappearance	–22.1 ± 1.4 a	–15.5 ± 2.2 b	–13.4 ± 1.1 b	–3.8 ± 1.2 c	5.4 ± 1.4 d	0.96**

[a] Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA. Data were also analyzed with linear regression relative to the degree of substitution of FO with the blended vegetable oil; the coefficient of determination R^2 and P values (*, <0.05; **, <0.01; and ***, <0.001) are reported. [b] See **Table 1** for diet abbreviations. Blank cells, not detected.

whole cells (i.e., hepatocytes) are isolated and incubated with labeled EFAs, i.e., (1-^{14}C) 18:2 n-6 or (1-^{14}C) 18:3 n-3. The use of this method is costly and time-consuming and can result in considerable variation in desaturase activity between species, not to mention within the same species (*8, 10*). The present study employed the whole-body fatty acid balance method, an in vivo method (*9*), which provides an estimation of an organism's overall capacity to metabolize EFAs within the context of an integrated system (*8*). The whole-body fatty acid balance method is a simple, relatively inexpensive, routine laboratory technique that generates consistent results between applications. To date, to the best of the authors' knowledge, this is the second instance in which this technique has been applied in a fish nutrition study. Turchini et al. (*10*) investigated the aptitude of Murray cod in elongating and desaturating 18:2 n-6 and 18:3 n-3 to higher homologues when fed diets containing either canola or linseed oil. The chief observation in this study was that Murray cod exhibited elongase, Δ-5, and Δ-6 desaturase activities, all of which were quantified using the whole-body fatty acid balance method. Given that the only difference between Turchini et al. (*10*) and the present study was the oil source and a small difference with regard to the size of initial fish, there was a good agreement between the results of the two studies with respect to the fate of consumed 18:1 n-9, 18:2 n-6, and 18:3 n-3, as well as the total elongase and desaturase activities in mmol/g of fish/day. Likewise, with respect to elongase and desaturase activities, the results obtained from this study are in general agreement and within the highly variable range of results obtained using the radiolabeled hepatocyte method (*25*, *26*). Additionally, the present study supports the peculiar and contrasting inverse order of accumulation and oxidation of 18:1 n-9, 18:2 n-6, and 18:3 n-3 exhibited by Murray cod, initially described by De Silva et al. (*27*) and later supported by Turchini et al. (*10*). Moreover, the occurrence of a "Δ-6 desaturase block" acting on 18:2 n-6 in the presence of excess 18:3 n-3, previously reported for Atlantic salmon (*24*), was not evident for Murray cod given the appearance of desaturase intermediates on the 18:2 n-6 biosynthetic pathway

Table 6. n-6 Fatty Acid Balance for the Normal Pathway of Juvenile Murray Cod Fed Different Experimental Diets over a 98 Day Period; Data Represent the Total Amount of Fatty Acid (mg) Per Fish[a]

	dietary treatments[b]					
	FO	VO25	VO50	VO75	VO100	R^2
			18:2 n-6			
intake	328.6 ± 9.6 a	416.6 ± 14.9 b	468.0 ± 27.5 b	568.8 ± 36.2 c	666.2 ± 35.0 d	0.99***
excretion	12.6 ± 1.3 a	17.2 ± 0.7 ab	18.7 ± 2.2 b	30.0 ± 1.7 c	32.8 ± 1.6 c	0.94**
initial body content	56.8 ± 0.6	57.3 ± 0.8	57.3 ± 0.9	57.1 ± 0.5	57.8 ± 1.5	0.58
final body content	262.7 ± 15.3 a	320.3 ± 17.1 ab	370.6 ± 22.6 bc	426.6 ± 35.0 c	445.2 ± 27.7 c	0.98**
accumulation	205.9 ± 15.5 a	263.0 ± 16.3 ab	313.3 ± 23.4 bc	369.5 ± 35.4 c	387.4 ± 27.1 c	0.98**
appearance/disappearance	–110.0 ± 8.5 b	–136.5 ± 9.0 b	–135.9 ± 20.2 b	–169.4 ± 2.6 b	–246.0 ± 26.1 a	0.84*
			18:3 n-6			
intake	8.4 ± 0.3 c	8.6 ± 0.3 c	4.3 ± 0.3 b	1.1 ± 0.1 a	1.1 ± 0.1 a	0.89*
excretion	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.1			0.87*
initial body content	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	0.58
final body content	10.8 ± 1.4	11.0 ± 1.9 a	10.2 ± 2.5 a	14.4 ± 3.4 a	28.2 ± 5.6 b	0.63
accumulation	8.5 ± 1.4 a	8.7 ± 1.8 a	7.9 ± 2.5 a	12.0 ± 3.4 a	25.9 ± 5.6 b	0.63
appearance/disappearance	0.4 ± 1.6 a	0.2 ± 1.4 a	3.7 ± 2.7 a	10.9 ± 3.4 a	24.7 ± 5.6 b	0.83*
			20:3 n-6			
intake	11.7 ± 0.3 c	6.5 ± 0.2 b	7.1 ± 0.4 b	2.5 ± 0.2 a		0.93**
excretion	0.6 ± 0.4	0.1 ± 0.1				0.63
initial body content	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	0.58
final body content	10.2 ± 0.8	9.1 ± 0.8	8.4 ± 0.7	8.1 ± 0.8	9.0 ± 0.6	0.45
accumulation	8.3 ± 0.8	7.2 ± 0.8	6.5 ± 0.7	6.3 ± 0.8	7.1 ± 0.6	0.46
appearance/disappearance	–2.7 ± 0.1 a	0.8 ± 0.6 b	–0.6 ± 0.4 b	3.7 ± 0.7 c	7.1 ± 0.6 d	0.87*
			20:4 n-6			
intake	48.1 ± 1.4 d	34.5 ± 1.2 c	23.0 ± 1.4 b	13.1 ± 0.8 a		0.99***
excretion	1.8 ± 0.2 c	1.2 ± 0.1 b	0.5 ± 0.3 a	0.4 ± 0.2 a		0.93**
initial body content	9.0 ± 0.1	9.1 ± 0.1	9.1 ± 0.1	9.1 ± 0.1	9.2 ± 0.2	0.58
final body content	40.8 ± 2.1 e	31.8 ± 1.3 d	23.9 ± 1.7 c	15.2 ± 0.8 b	7.8 ± 0.3 a	0.99***
accumulation	31.8 ± 2.2 e	22.7 ± 1.3 d	14.8 ± 1.9 c	6.1 ± 0.8 b	–4.6 ± 0.1 a	0.99***
appearance/disappearance	–14.5 ± 0.8 a	–10.6 ± 1.3 b	–7.8 ± 1.1 c	–6.7 ± 0.4 c	–1.4 ± 0.1 d	0.96**
			22:4 n-6			
intake	14.6 ± 0.4 d	12.0 ± 0.4 c	9.1 ± 0.5 b	4.1 ± 0.3 a		0.98***
excretion	0.6 ± 0.3		0.1 ± 0.1	0.3 ± 0.1		0.35
initial body content	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	0.58
final body content	11.0 ± 0.6 d	8.1 ± 0.7 c	5.8 ± 0.3 b	4.9 ± 0.8 b	1.6 ± 0.8 a	0.98**
accumulation	9.2 ± 0.6 d	6.3 ± 0.6 c	4.0 ± 0.3 b	3.1 ± 0.8 b	–0.8 ± 2.3 a	0.97**
appearance/disappearance	–4.8 ± 1.0 a	–5.7 ± 0.4 a	–5.0 ± 0.4 a	–0.8 ± 0.7 b	–0.3 ± 0.8 b	0.75

[a] Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA. Data were also analyzed with linear regression relative to the degree of substitution of FO with the blended vegetable oil; the coefficient of determination R^2 and P values (*, <0.05; **, <0.01; and ***, <0.001) are reported. [b] See **Table 1** for diet abbreviations. Blank cells, not detected.

(18:3 n-6). Taking all this into account, it is conceivable that the role of n-6 fatty acids in the general fatty acid balance of Murray cod is the result of an adaptation to a natural diet rich in freshwater crustaceans and ultimately n-6 fatty acids.

It has been outlined previously that dietary lipid source can have a marked impact on overall lipid metabolism, resulting in increased elongase and desaturase activities when a HUFA deficient vegetable oil is substituted for FO (*10*). Consistent with this observation, a significant increase in elongase/desaturase activities was evident in this study with the inclusion of the VO. Significantly greater proportions of 18:3 n-3 were elongated to dead end products as compared to that of 18:2 n-6, a result consistent with studies on Atlantic salmon (*28*). Furthermore, there was an appearance of fatty acids on both the Δ-9 (data not reported) and the Δ-6 biosynthetic pathways. This resulted in the production of fatty acids including 16:1 n-7, 18:4 n-3, and 18:3 n-6. In this instance, Δ-5 desaturase activity could not be quantified as an appearance of 20:5 n-3 and 20:4 n-6 was masked by concentrations of these fatty acids in the initial fish samples. However, it is probable that the production of these fatty acids would have been quantifiable if the experiment duration had been longer, although production would be negligible (*5*). The affinity of elongase and desaturase activities for fatty acids in the order of n-3 < n-6 < n-9 has been well-documented in teleost fish species (*7*, *10*) and is further supported in the present study. The Δ-6 desaturase has been previously described as the rate-limiting enzyme in the fatty acid biosynthetic pathway (*29*). However, elevated levels of 18:4 n-3 and 18:3 n-6 in Murray cod samples coupled with results from experiments in which diets were supplemented with oils rich in Δ-6 desaturated fatty acids (*30*) suggest that the production of n-3 and n-6 HUFA is more than likely limited by Δ-5 desaturase.

The occurrence of a positive feedback mechanism in relation to the amount of dietary precursor, or a negative feedback mechanism in response to decreased product, or possibly a combination of both, appears to be the driving influence behind the increase in elongase and desaturase in this experiment. Similar observations have been reported previously for Murray cod (*10*), as well as for other species (*31*). At this stage, the underlying mechanism responsible for increased activity is relatively unclear and requires further investigation. Tocher et al. (*32*) speculate that increased elongase and desaturase activities in fish are a direct result of a reduction in product rather than an increased provision of precursor for the first step in the biosynthetic pathway. Conversely, combining the results of the present study alongside those of Turchini et al. (*10*), we can speculate that at least for the species under examination, that increased activity is a result of elevated precursor level.

The whole-body fatty acid balance method has proved useful

Table 7. n-3 Fatty Acid Balance for the Normal Pathway of Juvenile Murray Cod Fed Different Experimental Diets over a 98 Day Period; Data Represent the Total Amount of Fatty Acid (mg) Per Fish[a]

	dietary treatments[b]					
	FO	VO25	VO50	VO75	VO100	R^2
			18:3 n-3			
intake	62.6 ± 1.8 a	452.9 ± 16.2 b	773.7 ± 45.5 c	1132.0 ± 72.1 d	1483.0 ± 77.8 e	0.99***
excretion	2.4 ± 0.2 a	13.7 ± 0.5 b	22.1 ± 2.8 c	46.0 ± 2.5 d	56.3 ± 2.5 e	0.97**
initial body content	10.1 ± 0.1	10.2 ± 0.2	10.2 ± 0.2	10.1 ± 0.1	10.3 ± 0.3	0.58
final body content	47.8 ± 1.0 a	268.5 ± 15.5 b	466.6 ± 38.8 c	633.8 ± 64.3 d	687.4 ± 48.5 d	0.96**
accumulation	37.7 ± 0.9 a	258.4 ± 15.4 b	456.4 ± 39.0 c	623.7 ± 64.4 d	677.1 ± 48.4 d	0.96**
appearance/disappearance	–22.5 ± 1.0 e	–180.8 ± 4.5 d	–295.2 ± 34.9 c	–462.3 ± 14.3 b	–749.6 ± 55.9 a	0.97**
			18:4 n-3			
intake	90.3 ± 2.6 e	65.0 ± 2.3 d	42.3 ± 2.5 c	23.6 ± 1.5 b	9.3 ± 0.5 a	0.99***
excretion	1.5 ± 0.7	1.2 ± 0.2	0.5 ± 0.3	0.1 ± 0.1		0.95**
initial body content	12.6 ± 0.14	12.7 ± 0.2	12.7 ± 0.2	12.6 ± 0.1	12.8 ± 0.3	0.58
final body content	56.8 ± 2.8 a	53.1 ± 1.6 a	54.9 ± 3.8 a	64.5 ± 2.7 a	102.7 ± 6.0 b	0.62
accumulation	44.3 ± 2.8 a	40.4 ± 1.4 a	42.3 ± 4.0 a	51.9 ± 2.8 a	89.9 ± 5.9 b	0.62
appearance/disappearance	–44.5 ± 1.6 a	–23.5 ± 1.5 b	0.4 ± 3.1 c	28.5 ± 1.4 d	80.5 ± 5.6 e	0.96**
			20:4 n-3			
intake	79.0 ± 2.3 e	55.0 ± 2.0 d	32.1 ± 1.9 c	16.3 ± 1.1 b	1.0 ± 0.1 a	0.99***
excretion	2.0 ± 0.2 b	1.6 ± 0.1 b	0.8 ± 0.2 a	0.4 ± 0.2 a		0.98***
initial body content	7.9 ± 0.1	7.9 ± 0.1	8.0 ± 0.1	7.9 ± 0.1	8.0 ± 0.2	0.58
final body content	59.2 ± 3.4 b	48.7 ± 2.3 b	44.1 ± 2.9 a	46.3 ± 3.3 a	61.7 ± 3.7 a	0.00
accumulation	51.3 ± 3.4 b	40.7 ± 2.2 a	36.1 ± 3.0 a	38.4 ± 3.3 a	53.6 ± 3.6 b	0.00
appearance/disappearance	–25.7 ± 2.0 a	–12.7 ± 1.3 b	4.8 ± 2.4 c	22.4 ± 2.2 d	52.6 ± 3.5 e	0.97**
			20:5 n-3			
intake	576.9 ± 16.9 e	395.5 ± 14.1 d	244.7 ± 14.4 c	137.1 ± 8.7 b	28.8 ± 1.5 a	0.99***
excretion	8.3 ± 0.9 d	6.6 ± 0.2 c	4.4 ± 0.6 b	3.5 ± 0.1 b	1.6 ± 0.1 a	0.99***
initial body content	55.2 ± 0.6	55.7 ± 0.8	55.7 ± 0.9	55.4 ± 0.5	56.2 ± 1.4	0.58
final body content	262.6 ± 16.2 e	186.8 ± 8.9 d	122.6 ± 6.0 c	77.9 ± 4.6 b	44.0 ± 0.3 a	0.98**
accumulation	207.4 ± 16.6 e	131.1 ± 8.2 d	67.0 ± 6.5 c	22.5 ± 4.9 b	–40.3 ± 3.9 a	0.99***
appearance/disappearance	–361.1 ± 10.5 a	–257.7 ± 9.4 b	–173.3 ± 12.4 c	–111.1 ± 4.3 d	–39.4 ± 2.7 e	0.99***
			22:5 n-3			
intake	260.2 ± 7.6 e	175.4 ± 6.3 d	107.6 ± 6.3 c	55.9 ± 3.6 b	6.1 ± 0.3 a	0.99***
excretion	6.9 ± 0.9 d	5.0 ± 0.2 c	3.3 ± 0.5 b	2.5 ± 0.2 b	0.8 ± 0.1 a	0.98***
initial body content	36.3 ± 0.4	36.7 ± 0.5	36.7 ± 0.6	36.5 ± 0.3	37.0 ± 0.9	0.58
final body content	264.3 ± 16.8 e	190.0 ± 9.0 d	138.2 ± 8.6 c	90.3 ± 7.0 b	55.9 ± 1.4 a	0.98**
accumulation	227.9 ± 17.0 e	153.3 ± 8.5 d	101.5 ± 9.0 c	53.8 ± 7.2 b	19.0 ± 1.6 a	0.98**
appearance/disappearance	–25.4 ± 11.8 a	–17.1 ± 5.9 a	–2.8 ± 7.4 ab	0.3 ± 4.0 ab	13.7 ± 1.6 c	0.98**
			22:6 n-3			
intake	828.7 ± 24.2 e	563.2 ± 20.1 d	348.8 ± 20.5 c	190.9 ± 12.2 b	38.5 ± 2.0 a	0.98***
excretion	18.6 ± 2.2 d	14.1 ± 0.3 c	9.3 ± 1.3 b	7.7 ± 0.6 b	3.2 ± 0.3 a	0.98**
initial body content	105.9 ± 1.2	106.9 ± 1.6	106.9 ± 1.7	106.5 ± 0.9	107.8 ± 2.7	0.58
final body content	681.9 ± 47.8 e	521.2 ± 28.1 d	371.3 ± 19.1 c	249.6 ± 14.4 b	129.8 ± 0.3 a	0.99***
accumulation	576.0 ± 48.5 e	414.3 ± 26.9 d	264.4 ± 20.3 c	143.1 ± 15.0 b	22.0 ± 2.8 a	0.99***
appearance/disappearance	–234.2 ± 33.9 a	–134.8 ± 21.6 b	–75.0 ± 17.3 bc	–40.1 ± 4.0 c	–13.3 ± 4.5 c	0.93**

[a] Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA. Data were also analyzed with linear regression relative to the degree of substitution of FO with the blended vegetable oil; the coefficient of determination R^2 and P values (*, <0.05; **, <0.01; and ***, <0.001) are reported. [b] See **Table 1** for diet abbreviations. Blank cells, not detected.

in answering key questions regarding the lipid metabolism of Murray cod. As with any method, there are certain limitations that can restrict accuracy and feasibility. The allowance for eicosanoid production, fatty acid derivatives chiefly from the C_{20} PUFA 20:3 n-6, 20:4 n-6, and 20:5 n-3 (*7*), is one variable that the whole-body fatty acid balance method does not take into consideration. However, as outlined previously (*9*, *10*), it is acceptable that the conversion of these C_{20} PUFA is minimal, having little impact on the total balance of fatty acids. Additionally, the implementation of the whole-body fatty acid balance method requires the use of a semipurified diet in order to minimize the underestimation of activity arising from the presence of elongase and desaturase intermediates present in a practical diet. This, coupled with a relatively long experimental period, limits the method to small scale laboratory trials.

In conclusion, using the whole-body fatty acid balance method, this study has confirmed that Murray cod efficiently elongate and desaturate 18:2 n-6 and 18:3 n-3. Dietary vegetable oil inclusion resulted in increased elongase and desaturase activities and exhibited a higher affinity toward n-3 fatty acids. The Δ-6 desaturase enzyme was found to be more active than both elongase and Δ-5 desaturase activity, while Murray cod was found to have a preferential order of fatty acid oxidation of 18:3 n-3 > 18:2 n-6 > 18:1 n-9. However, despite increased levels of elongase and desaturase activities, substituting dietary FO with a VO resulted in decreased growth rates, a substantial fillet fatty acid modification, and ramifications from a human health point of view. Ultimately, this study has clearly proven the usefulness of in vivo methods for the assessment of fatty acid metabolism, demonstrating good consistency between applications and comparability with results obtained using ex vivo counterparts.

ABBREVIATIONS USED

VO, vegetable oil blend; FO, fish oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsatu-

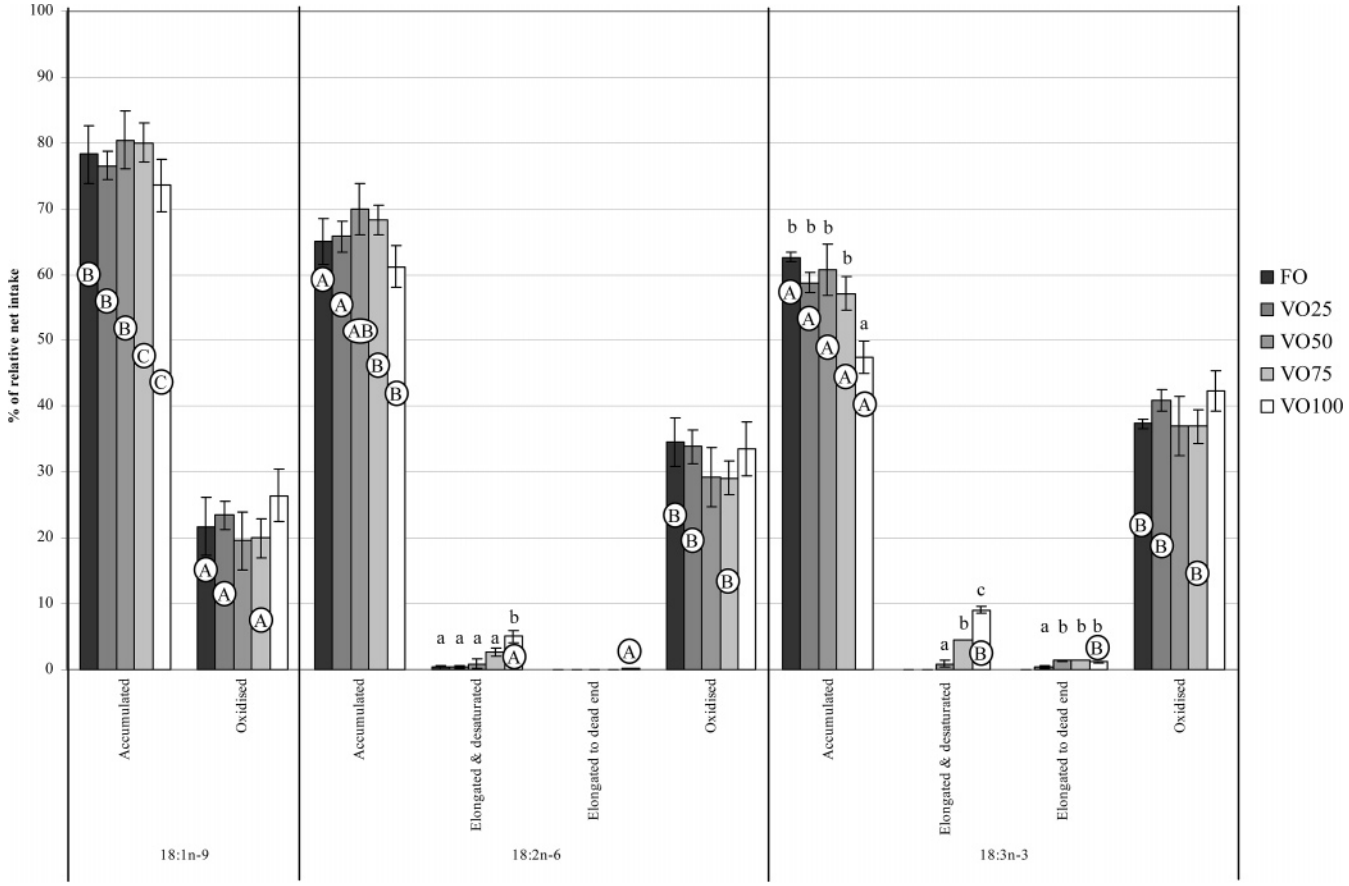

Figure 1. 18:1 n-9, 18:2 n-6, and 18:3 n-3 total balance of juvenile Murray cod fed different experimental diets over a 98 day period. Data represent the percentage of the total 18:1 n-9, 18:2 n-6, and 18:3 n-3 net intake, respectively. Lowercase superscripts represent statistically significant differences in fatty acid metabolism between dietary groups. Within each dietary treatment, different letters (circled, uppercase) indicate statistical differences between fatty acid accumulation, oxidation, elongation and desaturation, and elongation to dead end products (ANOVA and Student–Newman–Keuls posthoc test).

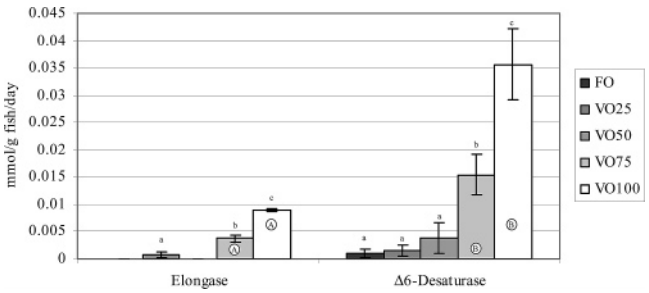

Figure 2. Elongase and Δ6-desaturase activity of 18:2 n-6 (mmol Σ of end products per gram of fish per day) in juvenile Murray cod fed different experimental diets over a 98 day period. Between each dietary treatment, different superscript letters (lowercase) indicate statistical differences (ANOVA and Student–Newman–Keuls posthoc test). Within each dietary group, uppercase, circled superscripts indicate statistically significant differences between enzyme activities.

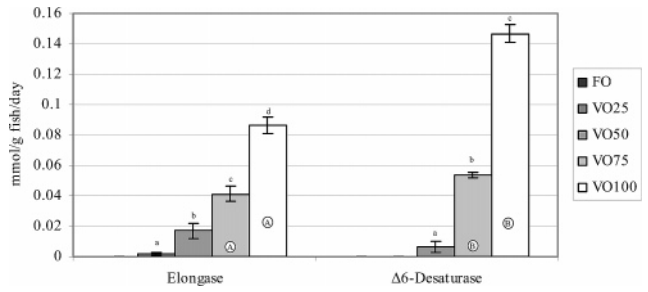

Figure 3. Elongase and Δ6-desaturase activity of 18:3 n-3 (mmol Σ of end products per gram of fish per day) in juvenile Murray cod fed different experimental diets over a 98 day period. Between each dietary treatment, different superscript letters (lowercase) indicate statistical differences (ANOVA and Student–Newman–Keuls posthoc test). Within each dietary group, uppercase, circled superscripts indicate statistically significant differences between enzyme activities.

rated fatty acids; HUFA, highly unsaturated fatty acids; EFA, essential fatty acids.

ACKNOWLEDGMENT

We thank Dane Newman, Bob Collins, and the staff of the Science, Technology and Innovation Initiative (STI) for technical assistance throughout the project.

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Received for review July 27, 2006. Revised manuscript received November 2, 2006. Accepted December 8, 2006.

JF062153X