

Apparent in Vivo Δ -6 Desaturase Activity, Efficiency, and Affinity Are Affected by Total Dietary C₁₈ PUFA in the Freshwater Fish Murray Cod

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Dietary fatty acids are known to modulate fatty acid metabolism in fish. However, the innate capability of fish to bioconvert short chain fatty acids to health promoting long chain fatty acids (LCPUFA) is insufficient to compensate for a reduced dietary intake. While many studies have focused on the dietary regulation of the fatty acid bioconversion pathways, there is little known regarding the effects of the dietary levels of C₁₈ polyunsaturated fatty acids (PUFA) on fatty acid metabolism. Here, we show a greater degree of apparent enzyme activity (Δ -6 desaturase) in fish fed a diet with higher amounts of dietary C₁₈ PUFA. In particular, fish receiving high amounts of dietary C₁₈ PUFA had a greater amount of Δ -6 desaturase activity acting on 18:3n-3 than 18:2n-6. However, with the gradual reduction of dietary C₁₈ PUFA there was a shift in substrate preference of Δ -6 desaturase from 18:3n-3 to 18:2n-6. This information will provide valuable insight for the implementation of low fish oil diets, which permit the maintenance of n-3 LCPUFA levels in farmed Murray cod.

KEYWORDS: *Maccullochella peelii peelii*; enzyme affinity; Δ -6 desaturase; fatty acid; linoleic acid; α -linolenic acid

INTRODUCTION

Unsustainable fishing practices and the resultant decline of global fish landings (1) have placed a heavy emphasis on aquaculture to meet the shortfalls in the supply of fish and seafood (2). Fish are known to be the primary readily available source of health promoting omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) for human consumption (3), and with this realization, the global consumption and demand for fish products is on the rise (4). However, the utilization of fish oil is necessary for the production of n-3 LCPUFA rich farmed fish (5), and this commodity is currently derived only from wild fisheries (6).

It has been widely demonstrated that the dietary fatty acid composition is mirrored in fish tissues (7, 8). Consequently, the simple direct substitution of fish oil in aquafeeds with readily available terrestrial alternatives, which are lacking in n-3 LCPUFA, will have a detrimental impact on the nutritional quality of the final farmed product (9).

Fish, as with all vertebrates, are capable of bioconverting dietary α -linolenic acid (18:3n-3, ALA), found in some vegetable oils, into health promoting n-3 LCPUFA (10). However, fish metabolism has adapted to the abundance of n-3 LCPUFA available in the aquatic food web, resulting in a limited efficacy of this metabolic pathway (5, 10). Consequently, despite the issues surrounding the sustainability and ethicality of the use of fish oil

(11) and its ever-rising price (US\$ 1,800 per tonne in July 2008 (12)), it remains widely utilized for aquafeed production (6) and as a result jeopardizes the future economic and sustainable development of the sector. However, it is envisaged that a solution can be achieved through a better understanding of fish lipid and fatty acid metabolism.

In vivo, 18:3n-3 (ALA) and 18:2n-6 (linoleic acid, LA) are bioconverted to n-3 LCPUFA and n-6 LCPUFA, respectively (i.e., docosahexaenoic acid; 22:6n-3 and arachidonic acid; 20:4n-6) via an alternating succession of desaturation and elongation enzymatic steps, involving three main enzymes: the elongase, the Δ -6 desaturase, and the Δ -5 desaturase (13). When fish are fed a diet lacking enzyme products (n-3 LCPUFA), the transcription rate and activity of the enzymes involved in the fatty acid desaturation and elongation pathway are increased (14, 15). However, it is also known that the rate of conversion of 18:3n-3 to n-3 LCPUFA is affected by the fatty acid composition of the diet. In particular, the presence of linoleic acid (18:2n-6) has been demonstrated to compete with 18:3n-3 for the desaturase and elongase enzymes (10, 16).

Despite the global research focus on the dietary regulation of the fatty acid bioconversion pathways, little is known regarding the effects of the dietary levels of C₁₈ polyunsaturated fatty acids (PUFA). Therefore, the aim of the present study was to evaluate the effects of graded amounts of dietary C₁₈ PUFA, with a constant ratio of 18:3n-3/ 18:2n-6 (1/1), and tentatively evaluate the presence of a feedback mechanism affecting desaturase and elongase enzyme activity. In consideration of its documented nutritional requirements and response to dietary

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fatty acid modification (7, 17, 18), the hypothesis was tested on the freshwater fish Murray cod (*Maccullochella peelii peelii*), an emerging species in the Australian aquaculture industry.

MATERIALS AND METHODS

Animals, Husbandry, and Experimental Diets. Murray cod [*M. peelii peelii* (Mitchell); order Perciformes; family Percichthyidae] of the 2006 year class were obtained from the Deakin University Murray cod hatchery (Warrnambool, VIC, Australia). Fish were graded to ensure homogeneity of size (~150 mm), and 540 juvenile specimens (mean weight 70.08 g ± 0.42) were individually weighed and randomly allocated to 18 tanks (30 fish per tank) in the Deakin University Aquaculture Research Centre. The husbandry facilities and management practices were the same as those previously described (18). Briefly, fish were housed in a multiple 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 °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.

Six iso-proteic, iso-lipidic experimental diets, varying only in lipid source, were formulated to contain a limited amount of fishmeal. The lipid sources, two vegetable oil blends, were formulated to contain a

constant 18:3n-3/18:2n-6 ratio of 1/1 with a varying total amount of C₁₈ PUFA, and subsequently named high polyunsaturated fatty acid vegetable oil (HPVO; 58.33% linseed oil and 41.67% sunflower oil) and low polyunsaturated fatty acid vegetable oil (LPVO; 14.95% linseed oil and 85.05% palm oil). Five experimental diets were then formulated with a graded inclusion of the two vegetable oil blends, in 25% increments, and named T1 (100% HPVO), T2 (75% HPVO and 25% LPVO), T3 (50% HPVO and 50% LPVO), T4 (25% HPVO and 75% LPVO), and T5 (100% LPVO). A fish oil based control diet (CD) was also used in addition to the dietary treatments (Table 1). The nutritional contents of the diets were based on previous findings (19) and prepared and stored as also reported previously (17). Fish were fed twice daily to apparent satiation at 1000 and 1700 h for 133 days, and weekly food consumption was recorded. Feces were collected between days 78 and 82. At day 134, all fish were anaesthetized and individually weighed, and 10 fish from each tank were randomly selected and euthanized and stored at -20 °C prior to chemical analysis.

Chemical Analyses. The nutrient composition of the experimental diets and whole fish samples was determined via proximate composition analysis according to standard procedures described previously (16, 20). Fatty acid analysis was performed on triplicate subsamples of each of the experimental diets and on three pooled whole body samples from each of the replicates. Following the lipid extraction (21), fatty acids were esterified into methyl esters using the acid catalyzed methylation method and analyzed by gas chromatography as previously described in detail

Table 1. Formulation and Proximate Composition of Experimental Diets, and Main Growth Performances and Feed Utilization Parameters of Juvenile Murray Cod Fed the Six Dietary Treatments for 133 Days

	dietary treatments ^a					
	CD	T1	T2	T3	T4	T5
Diet Formulation (mg g ⁻¹)						
fishmeal ^b	231.3	231.3	231.3	231.3	231.3	231.3
soybean meal ^b	231.3	231.3	231.3	231.3	231.3	231.3
wheat gluten ^c	231.3	231.3	231.3	231.3	231.3	231.3
wheat flour ^d	150.3	150.3	150.3	150.3	150.3	150.3
CMC ^e	10.0	10.0	10.0	10.0	10.0	10.0
Cr ₂ O ₃ ^f	3.0	3.0	3.0	3.0	3.0	3.0
Vit+min ^b	2.0	2.0	2.0	2.0	2.0	2.0
choline bitartrate ^f	2.0	2.0	2.0	2.0	2.0	2.0
fish oil ^b	138.8					
HPVO ^g		138.8	104.1	69.4	34.7	
LPVO ^h			34.7	69.4	104.1	138.8
Proximate (mg g ⁻¹)						
moisture	48.4	39.6	41.8	43.6	46.3	45.4
protein	483.4	484.1	481.0	477.0	480.6	479.2
lipid	178.4	182.9	173.8	185.2	178.2	171.1
ash	61.0	62.4	62.7	61.8	60.5	60.7
NFE ⁱ	228.8	230.9	240.8	232.3	234.4	243.5
Growth Performance and Feed Utilization ^j						
initial weight (g)	70.4 ± 0.0	69.5 ± 0.5	70.7 ± 1.9	70.2 ± 0.1	69.8 ± 1.1	69.9 ± 1.1
final weight (g)	168.9 ± 2.8 b	141.9 ± 6.9 a	142.3 ± 1.0 a	154.0 ± 3.1 a	145.4 ± 2.9 a	142.6 ± 5.2 a
gain%	140.1 ± 4.0 b	104.0 ± 8.6 a	101.6 ± 6.6 a	119.2 ± 4.4 a	108.3 ± 2.7 a	103.8 ± 5.6 a
SGR (% day ⁻¹) ^k	0.66 ± 0.01 b	0.53 ± 0.03 a	0.53 ± 0.03 a	0.59 ± 0.02 a	0.55 ± 0.01 a	0.53 ± 0.02 a
feed ration (%) ^l	1.11 ± 0.05	1.09 ± 0.05	1.15 ± 0.04	1.02 ± 0.02	1.16 ± 0.05	1.10 ± 0.02
FCR ^m	1.70 ± 0.10	2.06 ± 0.19	2.18 ± 0.12	1.75 ± 0.06	2.09 ± 0.01	2.05 ± 0.04

^a Diet abbreviations: CD, control diet; T1, 100% HPVO; T2, 75% HPVO and 25% LPVO; T3, 50% HPVO and 50% LPVO; T4, 25% HPVO and 75% LPVO; and T5, 100% LPVO. ^b Ridley Agriproducts, Narangba, Queensland, Australia. ^c Manildra, Auburn, New South Wales, Australia. ^d Black and Gold, Tooronga, Victoria, Australia. ^e Carboxymethyl cellulose: BDH Laboratory Supplies, Poole, United Kingdom. ^f Sigma-Aldrich, Inc. St. Louis, MO, USA. ^g HPVO: high PUFA vegetable oil blend; 58.33% linseed oil (Natures First, Cheltenham, Victoria, Australia) and 41.67% sunflower oil (Black and Gold, Tooronga, Victoria, Australia). ^h LPVO: low PUFA vegetable oil blend 14.95% linseed oil (Natures First, Cheltenham, Victoria, Australia) and 85.05% palm oil (Mel-Fry, MacQuarie Park, New South Wales, Australia). ⁱ NFE (nitrogen free extract): calculated by difference. ^j Values in the same row with the same letter are not significantly different ($P > 0.05$); data are reported as the mean ± SEM ($N = 3$). ^k SGR; specific growth rate = $[\ln(\text{final weight}) - \ln(\text{initial weight})] \times (\text{number of days})^{-1} \times 100$. ^l Feed ration % = (dry food fed per day) × $[(\text{final weight} - \text{initial weight})^{-1} \times 2^{-1}]$. ^m FCR; feed conversion ratio = (dry feed fed) × (wet weight gain)⁻¹.

(22). Fatty acid digestibility was measured using an inert chromium oxide marker and calculated using standard formulas (23).

Whole-Body Fatty Acid Balance Calculations. The in vivo assessment of fish fatty acid metabolism was deduced using the whole-body fatty acid balance method, which permits a reliable estimation of an organism's overall capability to metabolize fatty acids, as previously described in detail by Turchini et al. (24) and previously implemented on Murray cod (16, 18) and rainbow trout (*Oncorhynchus mykiss*) (25).

Statistical Analysis. All data were reported as means \pm standard error ($N = 3$). Data interpretation was based on two different statistical tests: data were analyzed among treatments by (i) linear or curvilinear regression (relative to dietary fatty acid composition 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 six experimental diets were iso-proteic (varying from 484 to 487 mg g⁻¹) and iso-lipidic (varying from 171 to 185 mg g⁻¹) (Table 1). During the 133 days of the feeding trial, fish roughly doubled their weight, and mortality was low and independent of the dietary treatments. Fish fed the CD diet grew significantly larger than all other treatments, recording greater final weight, gain %, and specific growth rate (SGR) ($P < 0.05$). Voluntary feed intake (feed ration %) and feed conversion ratio (FCR) were

unaffected by the dietary treatments (Table 1). The fatty acid compositions of the experimental diets are reported in Table 2. While the two vegetable oils blends (HPVO and LPVO) used to formulate the five graded diets had an 18:3n-3/18:2n-6 (ALA/LA) ratio close to 1, the resulting diets were varied in this respect. In fact, the ALA/LA ratio decreased from 0.9 in T1 to 0.7 in T5. In general and as expected, the CD had a higher level of eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). T1 recorded the highest PUFA content (67.5%), with 18:2n-6 and 18:3n-3 contributing 33.1% and 30.7%, respectively. The total PUFA content decreased gradually throughout the other treatments to 21.9% in T5 (11.3% of 18:2n-6 and 7.5% of 18:3n-3). In parallel with the decrease in PUFA, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) increased from 12.1% and 20.4% in T1 up to 41.8% and 36.3% in T5, respectively (Table 2).

Total lipid content of fish whole body was unaffected by the dietary treatments and increased from 12.2 mg g⁻¹ in initial fish to approximately 120 mg g⁻¹ at the end of the experiment. The dietary fatty acid makeup was mirrored in the fish body (Table 3). Fish fed the CD diet had the highest levels of EPA and DHA. T1 recorded the highest PUFA content (518 mg g⁻¹); however, the 18:2n-6 content was almost 1.6-fold higher than 18:3n-3 (236.2 and 148 mg g⁻¹, respectively). The total PUFA content in whole body samples gradually decreased to 268.9 mg g⁻¹ in fish receiving the T5 treatment, in which the 18:2n-6 content was 3-fold higher than 18:3n-3 (117.5 and 39 mg g⁻¹, respectively).

Table 2. Diet Fatty Acid Composition (mg per g of lipid) and Percentage Relative to Total Fatty Acids (% in Parentheses and Italics)

	diets ^a					
	CD	T1	T2	T3	T4	T5
14:0	62.6 (8.4)	4.7 (0.5)	6.5 (0.7)	7.5 (0.8)	9.3 (1.0)	11.6 (1.2)
16:0	170.0 (22.7)	68.7 (8.0)	135.8 (14.8)	189.5 (21.2)	252.9 (28.4)	341.5 (35.4)
18:0	30.7 (4.1)	24.5 (2.8)	31.6 (3.5)	32.5 (3.6)	36.4 (4.1)	44.5 (4.6)
20:0	2.4 (0.3)	1.6 (0.2)	2.0 (0.2)	2.2 (0.2)	2.6 (0.3)	2.9 (0.3)
22:0	2.2 (0.3)	3.1 (0.4)	2.8 (0.3)	2.5 (0.3)	1.8 (0.2)	1.2 (0.1)
24:0	1.4 (0.2)	1.7 (0.2)	2.1 (0.2)	1.6 (0.2)	2.4 (0.3)	1.5 (0.2)
16:1n-7	71.6 (9.6)	5.4 (0.6)	6.9 (0.8)	6.5 (0.7)	7.1 (0.8)	8.8 (0.9)
18:1n-9	81.4 (10.9)	152.7 (17.7)	201.4 (22.0)	224.3 (25.1)	257.6 (28.9)	311.7 (32.3)
18:1n-7	23.0 (3.1)	9.4 (1.1)	14.5 (1.6)	14.8 (1.7)	15.7 (1.8)	21.4 (2.2)
20:1	9.7 (1.3)	2.8 (0.3)	2.5 (0.3)	2.8 (0.3)	3.2 (0.4)	3.5 (0.4)
22:1	7.1 (0.9)	2.3 (0.3)	2.7 (0.3)	1.9 (0.2)	3.1 (0.4)	3.1 (0.3)
24:1n-9	3.8 (0.5)	3.5 (0.4)	3.0 (0.3)	3.1 (0.3)	3.8 (0.4)	1.8 (0.2)
18:2n-6	43.2 (5.8)	285.1 (33.1)	249.9 (27.3)	201.6 (22.6)	153.1 (17.2)	109.5 (11.3)
18:3n-6	2.1 (0.3)	0.4 (0.0)	0.3 (0.0)	0.5 (0.1)	0.0 (0.0)	0.3 (0.0)
20:2n-6	1.5 (0.2)	0.7 (0.1)	1.0 (0.1)	0.7 (0.1)	0.4 (0.0)	0.4 (0.0)
20:3n-6	1.7 (0.2)	0.1 (0.0)	0.4 (0.0)	0.2 (0.0)	0.2 (0.0)	0.1 (0.0)
20:4n-6	8.7 (1.2)	2.0 (0.2)	1.9 (0.2)	1.9 (0.2)	1.7 (0.2)	1.5 (0.2)
22:2n-6	4.5 (0.6)	0.4 (0.0)	0.6 (0.1)	0.4 (0.0)	0.5 (0.1)	0.3 (0.0)
22:4n-6	1.5 (0.2)	0.2 (0.0)	0.2 (0.0)	0.2 (0.0)	0.3 (0.0)	0.5 (0.0)
18:3n-3	10.9 (1.5)	263.9 (30.7)	223.5 (24.4)	170.4 (19.1)	112.8 (12.6)	72.6 (7.5)
18:4n-3	13.8 (1.8)	1.1 (0.1)	1.4 (0.2)	1.5 (0.2)	1.4 (0.2)	1.5 (0.2)
20:3n-3	1.3 (0.2)	0.3 (0.0)	0.5 (0.1)	0.3 (0.0)	0.2 (0.0)	0.2 (0.0)
20:4n-3	6.2 (0.8)	0.5 (0.1)	0.8 (0.1)	0.5 (0.1)	0.5 (0.1)	0.6 (0.1)
20:5n-3	99.2 (13.3)	8.1 (0.9)	7.7 (0.8)	8.1 (0.9)	6.8 (0.8)	7.0 (0.7)
22:5n-3	16.7 (2.2)	1.9 (0.2)	1.8 (0.2)	1.9 (0.2)	1.8 (0.2)	2.1 (0.2)
22:6n-3	70.1 (9.4)	16.0 (1.9)	13.8 (1.5)	15.7 (1.8)	15.6 (1.8)	14.7 (1.5)
SFA	269.3 (36.0)	104.2 (12.1)	180.8 (19.7)	235.8 (26.4)	305.4 (34.3)	403.2 (41.8)
MUFA	196.6 (26.3)	175.9 (20.4)	231.1 (25.2)	253.4 (28.4)	290.6 (32.6)	350.3 (36.3)
PUFA	281.4 (37.7)	580.7 (67.5)	503.7 (55.0)	403.9 (45.2)	295.5 (33.1)	211.1 (21.9)
n-6 PUFA	63.2 (8.5)	288.8 (33.6)	254.2 (27.8)	205.5 (23.0)	156.4 (17.5)	112.4 (11.7)
n-3 PUFA	218.2 (29.2)	291.8 (33.9)	249.5 (27.2)	198.5 (22.2)	139.1 (15.6)	98.7 (10.2)
ALA+LA ^b	54.1 (7.3)	549.0 (63.8)	473.4 (51.7)	372.0 (41.7)	265.9 (29.8)	182.1 (18.8)
ALA/LA ^c	0.3	0.9	0.9	0.8	0.7	0.7

^a See Table 1 for diet abbreviations. ^b Sum of ALA (α -linolenic acid, 18:3n-3) and LA (linoleic acid, 18:2n-6). ^c Ratio of ALA (α -linolenic acid, 18:3n-3) to LA (linoleic acid, 18:2n-6).

Table 3. Lipid (mg g⁻¹ on Wet Basis) and Fatty Acid Composition (mg per g of Lipid) of Murray Cod Whole Bodies at the End of the Feeding Trial^a

	dietary treatments ^b						
	initial	CD	T1	T2	T3	T4	T5
lipid	12.2	132.9 ± 3.4	125.3 ± 4.2	130.7 ± 7.0	128.5 ± 11.3	115.3 ± 3.4	110.4 ± 4.3
14:0	35.2	56.5 ± 1.4 b	17.5 ± 1.2 a	18.5 ± 1.0 a	19.1 ± 1.3 a	19.9 ± 0.5 a	21.7 ± 0.8 a
16:0	136.4	189.2 ± 4.9 c	125.7 ± 2.5 a	151.1 ± 5.6 b	180.5 ± 1.5 c	211.7 ± 6.4 d	219.9 ± 3.6 d
18:0	29.9	33.3 ± 0.7	32.6 ± 1.0	32.0 ± 1.2	33.8 ± 0.1	34.6 ± 0.6	34.3 ± 0.6
20:0	1.1	1.4 ± 0.1 b	1.3 ± 0.0 ab	1.2 ± 0.0 a	1.3 ± 0.0 a	1.3 ± 0.0 ab	1.3 ± 0.0 ab
22:0	1.3	1.2 ± 0.1 bc	1.6 ± 0.1 d	1.5 ± 0.1 cd	1.3 ± 0.1 bcd	1.1 ± 0.1 ab	0.9 ± 0.1 a
24:0	0.9	2.2 ± 0.1	1.2 ± 0.1	1.5 ± 0.4	1.5 ± 0.5	1.6 ± 0.3	2.4 ± 0.1
16:1n-7	52.0	81.5 ± 2.4 b	26.4 ± 1.4 a	27.8 ± 1.5 a	27.7 ± 1.8 a	30.6 ± 1.7 a	31.3 ± 0.9 a
18:1n-9	175.6	133.0 ± 3.2 a	201.8 ± 2.4 b	218.1 ± 3.8 c	252.6 ± 2.7 d	276.7 ± 4.8 e	296.3 ± 0.9 f
18:1n-7	25.5	30.8 ± 0.7 c	20.9 ± 0.4 a	21.5 ± 1.0 a	23.9 ± 1.0 ab	26.3 ± 1.0 b	26.7 ± 0.4 b
20:1	13.0	9.9 ± 0.2 c	7.8 ± 0.3 a	8.1 ± 0.4 ab	8.2 ± 0.6 ab	8.4 ± 0.2 ab	9.3 ± 0.1 bc
22:1	5.4	4.5 ± 0.2 b	3.0 ± 0.1 a	2.9 ± 0.2 a	2.9 ± 0.2 a	3.1 ± 0.2 a	3.2 ± 0.1 a
24:1n-9	4.2	3.9 ± 0.4 b	2.4 ± 0.1 a	2.7 ± 0.3 ab	2.9 ± 0.5 ab	3.3 ± 0.3 ab	4.0 ± 0.2 b
18:2n-6	67.0	70.3 ± 2.4 a	236.2 ± 11.6 f	199.2 ± 1.3 e	185.4 ± 2.4 d	153.8 ± 2.1 c	117.5 ± 0.7 b
18:3n-6	2.3	3.2 ± 0.1 a	12.7 ± 0.9 e	11.0 ± 0.1 d	9.9 ± 0.3 cd	9.4 ± 0.4 c	6.6 ± 0.1 b
20:2n-6	1.6	1.7 ± 0.1 a	2.7 ± 0.1b c	2.3 ± 0.1 b	2.4 ± 0.0 bc	2.2 ± 0.1 b	2.1 ± 0.0 bc
20:3n-6	1.4	2.0 ± 0.1 a	2.7 ± 0.1 b	2.7 ± 0.0 b	2.7 ± 0.26 b	2.9 ± 0.1 b	2.8 ± 0.1 b
20:4n-6	7.7	9.4 ± 0.1 b	4.1 ± 0.3 a	4.2 ± 0.3 a	4.3 ± 0.4 a	4.1 ± 0.1 a	4.1 ± 0.5 a
22:2n-6	3.1	4.3 ± 0.1 b	1.3 ± 0.1 a	1.5 ± 0.1 a	1.5 ± 0.2 a	1.5 ± 0.1 a	1.5 ± 0.1 a
22:4n-6	1.4	2.0 ± 0.0 b	0.9 ± 0.1 a	1.0 ± 0.0 a	0.9 ± 0.1 a	0.9 ± 0.0 a	1.3 ± 0.3 a
18:3n-3	7.0	9.1 ± 0.3 a	148.4 ± 11.0 f	114.4 ± 1.3 e	97.4 ± 3.7 d	70.3 ± 0.9 c	39.0 ± 0.9 b
18:4n-3	8.0	12.3 ± 0.5 b	21.1 ± 1.4 e	17.7 ± 0.1 d	14.6 ± 0.4 c	12.1 ± 0.4 b	8.1 ± 0.1 a
20:3n-3	13.8	1.0 ± 0.1 a	4.4 ± 0.2 d	3.5 ± 0.1 c	3.6 ± 0.1 c	3.0 ± 0.1 c	2.1 ± 0.3 b
20:4n-3	5.2	7.6 ± 0.2 c	10.1 ± 0.5 e	8.8 ± 0.0 d	7.9 ± 0.2 c	6.9 ± 0.0 b	5.5 ± 0.1 a
20:5n-3	49.8	64.2 ± 1.3 b	21.2 ± 2.1 a	21.8 ± 1.4 a	21.6 ± 2.1 a	20.6 ± 0.2 a	21.6 ± 1.3 a
22:5n-3	28.4	40.2 ± 0.8 b	15.8 ± 1.1 a	15.4 ± 0.8 a	15.2 ± 1.2 a	15.3 ± 0.1 a	15.4 ± 0.9 a
22:6n-3	60.7	83.5 ± 0.9 b	36.9 ± 1.9 a	36.4 ± 0.7 a	38.2 ± 1.7 a	38.9 ± 0.3 a	41.2 ± 1.6 a
SFA	204.8	283.8 ± 6.5 d	179.8 ± 2.6 a	205.7 ± 7.5 b	237.5 ± 2.3 c	270.2 ± 6.9 d	280.5 ± 3.4 d
MUFA	275.7	263.6 ± 4.8 a	262.3 ± 0.2 a	281.1 ± 6.6 a	318.3 ± 5.4 b	348.3 ± 7.1 c	370.9 ± 2.1 d
PUFA	257.4	310.8 ± 6.0 b	518.5 ± 19.7 f	440.0 ± 2.9 e	405.6 ± 4.7 d	341.8 ± 4.0 c	268.9 ± 3.5 a
n-6 PUFA	84.6	92.9 ± 2.7 a	260.6 ± 11.9 e	221.9 ± 1.7 d	207.1 ± 2.4 d	174.8 ± 2.4 c	136.0 ± 0.5 b
n-3 PUFA	172.8	217.9 ± 3.7 d	258.0 ± 7.8 e	218.1 ± 1.8 d	198.5 ± 2.6 c	167.0 ± 1.7 b	133.0 ± 3.4 a
ALA+LA ^c	74	79.4 ± 2.0 a	384.6 ± 17.0 f	313.6 ± 2.0 e	282.8 ± 4.6 d	224.1 ± 2.3 c	156.5 ± 1.2 b
ALA/LA ^d	0.1	0.1 ± 0.0 a	0.6 ± 0.0 f	0.6 ± 0.0 e	0.5 ± 0.0 d	0.5 ± 0.0 c	0.3 ± 0.0 b

^a Data are reported as the mean ± S.E.M (N = 3). Values in the same row with the same letter are not significantly different ($P > 0.05$); initial sample was not included in the statistical analysis. ^b See **Table 1** for diet abbreviations. ^c Sum of ALA (α -linolenic acid, 18:3n-3) and LA (linoleic acid, 18:2n-6). ^d Ratio of ALA (α -linolenic acid, 18:3n-3) to LA (linoleic acid, 18:2n-6).

Consequently, the total ALA/LA ratio in whole body samples was markedly different from the dietary levels. Significantly higher levels of all fatty acids along the n-3 and n-6 elongation and desaturation pathways (namely, 18:3n-6, 18:4n-3, and 20:4n-3) were found in fish receiving higher levels of C₁₈ PUFA in their diets (**Table 3**).

The digestibility of 18:2n-6 was not affected by the dietary treatment ($P > 0.05$), while 18:3n-3 was digested to a significantly greater extent in T1 (98.3%) and progressively less with the increase of dietary C₁₈ PUFA (90.3% in T5) (**Table 4**). The same trend was apparent for total fatty acid digestibility and total lipid digestibility, which decreased from 96.3% and 92.4% in T1, to 81.8 and 72.5% in T5, respectively. Within the same treatment, the apparent digestibility of unsaturated fatty acids decreased in relation to the degree of unsaturation, while there was an apparent preference for fatty acids with shorter carbon chains over fatty acids with longer carbon chains (**Table 4**).

In **Table 5**, the key results of the whole-body fatty acid balance method are reported. The total disappearance of 18:2n-6 and 18:3n-3 was proportional to dietary intake, as was the case for the majority of fatty acids. In fish receiving T1, there was a greater appearance of fatty acids along the n-3 and n-6 desaturation and elongation pathways recorded. These appearances decreased

gradually with the decrease of dietary C₁₈ PUFA content and were significantly lower in fish receiving T5.

In fish fed the CD diet, the fatty acids recording the greatest amount of apparent β -oxidation were in decreasing order 20:5n-3, 16:0, 14:0, and 22:6n-3 (0.308, 0.271, 0.167, and 0.116 $\mu\text{mol g}^{-1} \text{day}^{-1}$, respectively) (**Table 6**). Apparent β -oxidation in the five experimental treatments was largely affected by the dietary fatty acid makeup. The β -oxidation of 16:0 and 18:1n-9 was lower in T1 (0.145 and 0.382 $\mu\text{mol g}^{-1} \text{day}^{-1}$, respectively) and higher in T5 (1.079 and 0.803 $\mu\text{mol g}^{-1} \text{day}^{-1}$, respectively), while inversely, the β -oxidation of 18:2n-6 and 18:3n-3 was higher in T1 (0.742 and 0.822 $\mu\text{mol g}^{-1} \text{day}^{-1}$, respectively) and lower in T5 (0.247 and 0.218 $\mu\text{mol g}^{-1} \text{day}^{-1}$, respectively) (**Table 6**).

Similarly, apparent *in vivo* Δ -6 desaturase activity was largely affected by the dietary treatments (**Figure 1**). In fish fed T1, the highest activity of apparent Δ -6 desaturase was recorded on 18:3n-3, 0.076 $\mu\text{mol g}^{-1} \text{day}^{-1}$, and only 0.044 $\mu\text{mol g}^{-1} \text{day}^{-1}$ on 18:2n-6. This activity decreased alongside the decrease in total dietary C₁₈ PUFA from T1 to T5 treatment. However, in fish receiving T5 the highest Δ -6 desaturase activity was recorded on 18:2n-6 (0.02 $\mu\text{mol g}^{-1} \text{day}^{-1}$ in comparison to 0.01 $\mu\text{mol g}^{-1} \text{day}^{-1}$ on 18:3n-3). Total apparent elongase activity was also partially affected by the dietary treatments (**Figure 1**). The specific

Table 4. Lipid and Fatty Acid Apparent Digestibility (%) in Murray Cod^a

	dietary treatments ^b					
	CD	T1	T2	T3	T4	T5
14:0	89.0 ± 0.99	92.1 ± 1.54	87.0 ± 0.97	84.0 ± 3.19	84.0 ± 0.18	78.5 ± 6.10
16:0	84.5 ± 1.74 a	92.3 ± 1.45 b	82.7 ± 1.35 a	80.5 ± 3.38 a	81.1 ± 0.64 a	82.5 ± 0.53 a
18:0	78.6 ± 2.46 a	89.1 ± 1.79 b	78.3 ± 2.19 a	75.9 ± 3.91 a	75.0 ± 0.83 a	76.1 ± 1.18 a
20:0	79.3 ± 1.31	85.6 ± 1.35	75.0 ± 2.28	73.8 ± 6.50	79.8 ± 0.98	76.0 ± 1.32
22:0	83.5 ± 1.44	85.9 ± 2.34	77.8 ± 3.22	82.8 ± 3.64	83.2 ± 1.09	72.5 ± 4.00
24:0	76.1 ± 1.45	77.2 ± 2.51	76.3 ± 1.31	80.6 ± 1.96	78.6 ± 2.27	78.6 ± 2.72
16:1n-7	95.1 ± 0.33 a	92.3 ± 1.84 ab	90.3 ± 1.66 ab	87.2 ± 3.84 ab	86.6 ± 0.89 ab	81.5 ± 4.75 b
18:1n-9	93.8 ± 0.30	96.3 ± 1.06	93.1 ± 0.73	91.3 ± 1.43	87.1 ± 0.06	89.5 ± 4.40
18:1n-7	92.3 ± 0.46	93.9 ± 1.92	91.0 ± 1.84	87.2 ± 3.00	83.8 ± 0.27	88.7 ± 3.34
20:1n-9	92.3 ± 0.38 ab	91.6 ± 1.73 b	85.0 ± 2.64 ab	83.5 ± 4.96 ab	85.6 ± 0.44 ab	78.3 ± 4.20 a
22:1n-9	88.2 ± 0.92	91.3 ± 1.91	86.6 ± 2.74	84.9 ± 2.36	86.1 ± 0.83	81.3 ± 1.75
24:1n-9	74.7 ± 2.14	82.0 ± 8.34	78.0 ± 1.76	77.3 ± 3.75	81.6 ± 2.29	69.5 ± 4.91
18:2n-6	97.0 ± 0.59	97.7 ± 0.96	97.3 ± 1.32	95.8 ± 2.23	92.5 ± 0.26	92.9 ± 3.31
18:3n-6	96.5 ± 1.08	80.8 ± 2.22	89.4 ± 5.31	85.8 ± 7.16	78.6 ± 2.72	77.8 ± 2.75
20:2n-6	93.8 ± 1.33	83.4 ± 3.96	90.6 ± 1.45	89.4 ± 3.64	77.6 ± 6.81	80.9 ± 1.78
20:3n-6	96.9 ± 0.84 b	72.8 ± 1.66 a	75.7 ± 4.60 a	68.6 ± 2.58 a	67.5 ± 2.13 a	71.1 ± 2.05 a
20:4n-6	96.4 ± 0.26 b	94.0 ± 1.84 b	87.8 ± 3.36 ab	88.1 ± 2.61 ab	89.3 ± 0.49 ab	80.9 ± 3.83 a
22:2n-6	98.2 ± 0.48	94.5 ± 5.51	98.4 ± 1.57	96.9 ± 3.14	99.2 ± 0.81	98.8 ± 1.15
22:4n-6	92.6 ± 1.73	95.0 ± 4.99	86.6 ± 6.69	95.9 ± 4.06	92.1 ± 7.86	87.9 ± 6.77
18:3n-3	96.1 ± 0.27 b	98.3 ± 0.70 b	96.9 ± 0.74 b	95.9 ± 1.11 b	94.0 ± 0.33 b	90.3 ± 2.12 a
18:4n-3	98.1 ± 0.44	93.4 ± 2.64	92.4 ± 1.40	86.2 ± 4.60	90.0 ± 1.55	91.4 ± 2.56
20:3n-3	88.3 ± 5.08	86.6 ± 3.44	85.4 ± 2.19	77.6 ± 5.63	83.2 ± 2.40	78.6 ± 2.72
20:4n-3	97.1 ± 0.13	83.2 ± 9.69	77.9 ± 9.12	86.5 ± 2.29	85.4 ± 4.20	85.2 ± 1.12
20:5n-3	98.7 ± 0.05	96.3 ± 1.13	95.2 ± 1.60	95.2 ± 1.59	94.3 ± 0.86	90.4 ± 3.66
22:5n-3	95.8 ± 0.72	92.7 ± 1.95	85.2 ± 1.55	82.0 ± 5.85	84.1 ± 2.53	82.8 ± 1.95
22:6n-3	97.0 ± 0.43 c	92.9 ± 1.17 b	88.6 ± 1.34 b	91.1 ± 1.53 b	88.3 ± 0.95 b	82.6 ± 1.98 a
TOT FA	91.3 ± 0.44 c	96.3 ± 0.90 d	92.2 ± 0.99 c	89.6 ± 1.97b c	85.9 ± 0.11 b	81.8 ± 2.13 a
lipid	86.7 ± 1.25b c	92.4 ± 1.26 c	85.2 ± 1.49 bc	84.5 ± 0.75b c	80.1 ± 1.10 b	72.5 ± 3.71 a

^a Data are reported as the mean ± S.E.M (N = 3). Values in the same row with the same letter are not significantly different ($P > 0.05$). ^b See **Table 1** for diet abbreviations.

Table 5. Individual Fatty Acid Appearance/Disappearance (mg per Fish) during the 133 Day Experimental Period^a

	dietary treatments ^b					
	CD	T1	T2	T3	T4	T5
14:0	-783.1 ± 135.3 a	-110.3 ± 7.8 b	-119.2 ± 19.5 b	-101.5 ± 41.5 b	-195.3 ± 22.2 b	-203.6 ± 5.3 b
16:0	-1,425.2 ± 421.3 c	-683.3 ± 198.2 c	-1,536.5 ± 185.6 c	-1,904.4 ± 327.5 c	-3,655.5 ± 430.0 b	-5,196.5 ± 116.7 a
18:0	-265.6 ± 68.4 a	-278.7 ± 57.6 a	-361.2 ± 39.9 a	-282.8 ± 67.4 a	-477.6 ± 55.9 ab	-614.7 ± 11.7 b
20:0	-37.6 ± 3.5 b	-22.9 ± 2.2 c	-29.9 ± 0.6 bc	-29.0 ± 2.0 bc	-48.7 ± 2.9 a	-46.5 ± 1.6 a
22:0	-42.3 ± 4.7 b	-57.8 ± 4.0 a	-46.0 ± 2.0 b	-43.8 ± 4.5 b	-37.2 ± 0.7 b	-20.0 ± 1.3 c
24:0	7.5 ± 14.4 b	-23.8 ± 4.3 ab	-24.5 ± 6.8 ab	-15.0 ± 9.5 ab	-37.4 ± 3.4 a	-1.2 ± 2.6 b
16:1n-7	-752.6 ± 193.3 a	-113.9 ± 13.6 b	-108.1 ± 32.1 b	-58.9 ± 62.3 b	-109.1 ± 50.0 b	-142.3 ± 11.9 b
18:1n-9	-919.5 ± 219.5 d	-1,996.0 ± 337.4 c	-2,759.9 ± 204.0 bc	-2,288.3 ± 461.0 bc	-3,468.4 ± 466.8 ab	-4,256.2 ± 127.0 a
18:1n-7	-192.0 ± 59.6 ab	-91.2 ± 22.2 b	-191.5 ± 28.4 ab	-108.7 ± 53.0 b	-162.7 ± 43.0 ab	-301.3 ± 4.1 a
20:1	-171.5 ± 12.9 a	-42.6 ± 5.5 b	-23.1 ± 6.9 b	-13.5 ± 21.3 b	-52.9 ± 6.1 b	-36.1 ± 3.4 b
22:1	-139.8 ± 13.7 a	-50.7 ± 3.9 bc	-60.0 ± 4.9 bc	-31.9 ± 9.6 c	-73.7 ± 3.8 b	-62.0 ± 1.5 bc
24:1n-9	-38.1 ± 4.2 b	-71.7 ± 4.6 a	-51.2 ± 5.1 ab	-46.2 ± 9.3 b	-71.6 ± 4.2 a	-5.8 ± 3.7 c
18:2n-6	-313.9 ± 130.9 c	-4,119.8 ± 554.8 a	-3,749.1 ± 196.8 a	-2,360.4 ± 309.5 b	-2,165.3 ± 264.9 b	-1,426.5 ± 60.4 b
18:3n-6	-12.1 ± 5.6 a	200.5 ± 31.8 c	176.0 ± 10.4 c	163.9 ± 17.2 c	136.9 ± 12.5 c	79.5 ± 7.0 b
20:2n-6	-20.7 ± 3.9 a	18.6 ± 1.4 c	3.1 ± 1.2 b	15.6 ± 5.3 c	12.7 ± 1.7 bc	12.2 ± 1.7 bc
20:3n-6	-20.1 ± 5.1 a	35.0 ± 4.7 b	30.0 ± 2.0 b	37.3 ± 4.7 b	32.8 ± 1.8 b	29.9 ± 2.0 b
20:4n-6	-117.7 ± 18.2 a	-45.7 ± 4.0 b	-35.8 ± 3.9 b	-28.2 ± 8.7 b	-42.1 ± 5.4 b	-34.6 ± 6.2 b
22:2n-6	-68.8 ± 10.2 a	-12.8 ± 2.1 b	-14.2 ± 2.0 b	-8.6 ± 5.2 b	-15.5 ± 2.7 b	-9.7 ± 0.6 b
22:4n-6	-8.6 ± 3.7	-2.6 ± 1.1	0.5 ± 0.3	-0.1 ± 1.9	-6.4 ± 0.5	-1.4 ± 6.1
18:3n-3	-185.2 ± 24.4 e	-4,643.3 ± 433.8 a	-4,054.8 ± 156.4 a	-2,750.7 ± 149.4 b	-2,005.0 ± 165.1 c	-1,188.8 ± 38.6 d
18:4n-3	-216.5 ± 34.8 a	282.3 ± 51.0 d	222.4 ± 14.0 cd	183.1 ± 22.8 cd	97.7 ± 15.3 bc	23.7 ± 4.6 b
20:3n-3	-132.3 ± 2.0 a	-45.7 ± 7.6 d	-65.6 ± 0.6 cd	-54.0 ± 9.3 cd	-73.8 ± 1.7 bc	-89.5 ± 4.5 b
20:4n-3	-61.0 ± 16.5 a	126.2 ± 21.7 d	101.5 ± 5.8 cd	99.7 ± 13.9 cd	58.6 ± 4.4 bc	29.9 ± 3.0 b
20:5n-3	-2,059.7 ± 184.1 a	-266.6 ± 15.9 b	-231.6 ± 24.3 b	-218.8 ± 58.8 b	-266.8 ± 17.1 b	-255.9 ± 10.9 b
22:5n-3	158.4 ± 66.6 b	-10.8 ± 5.8 a	-0.3 ± 14.6 a	14.3 ± 35.8 a	-29.9 ± 9.1 a	-46.8 ± 3.9 a
22:6n-3	-780.5 ± 159.2 a	-275.2 ± 26.6 b	-191.8 ± 15.1 b	-169.3 ± 69.6 b	-271.0 ± 37.5 b	-194.3 ± 3.3 b

^a Data are reported as the mean ± S.E.M (N = 3). Values in the same row with the same letter are not significantly different ($P > 0.05$). ^b See **Table 1** for diet abbreviations.

Table 6. Individual Apparent *in Vivo* Fatty Acid β -Oxidation (μmol of Fatty Acid Disappeared per Gram of Fish per Day; Mean \pm S.E.M; $N = 3$) of Selected Fatty Acids^a

	dietary treatments ^b					
	CD	T1	T2	T3	T4	T5
14:0	0.167 \pm 0.030 b	0.026 \pm 0.002 a	0.028 \pm 0.005 a	0.023 \pm 0.009 a	0.045 \pm 0.006 a	0.047 \pm 0.001 a
16:0	0.271 \pm 0.082 a	0.145 \pm 0.046 a	0.318 \pm 0.041 a	0.376 \pm 0.073 a	0.755 \pm 0.099 b	1.079 \pm 0.020 c
18:1n-9	0.158 \pm 0.038 a	0.382 \pm 0.073 b	0.518 \pm 0.042 b	0.414 \pm 0.088 b	0.651 \pm 0.095 bc	0.803 \pm 0.027 c
18:2n-6	0.054 \pm 0.023 a	0.742 \pm 0.131 c	0.669 \pm 0.041 c	0.390 \pm 0.066 b	0.373 \pm 0.057 b	0.247 \pm 0.014 ab
20:4n-6	0.019 \pm 0.003 b	0.008 \pm 0.001 a	0.006 \pm 0.001 a	0.005 \pm 0.002 a	0.007 \pm 0.001 a	0.005 \pm 0.000 a
18:3n-3	0.032 \pm 0.004 a	0.822 \pm 0.114 d	0.712 \pm 0.034 d	0.455 \pm 0.038 c	0.352 \pm 0.039 bc	0.218 \pm 0.006 b
20:5n-3	0.308 \pm 0.041 b	0.047 \pm 0.002 a	0.041 \pm 0.004 a	0.033 \pm 0.014 a	0.047 \pm 0.004 a	0.045 \pm 0.001 a
22:6n-3	0.116 \pm 0.024 b	0.045 \pm 0.005 a	0.031 \pm 0.003 a	0.026 \pm 0.011 a	0.044 \pm 0.007 a	0.032 \pm 0.001 a

^a Values in the same row with the same letter are not significantly different ($P > 0.05$). ^b See Table 1 for diet abbreviations.

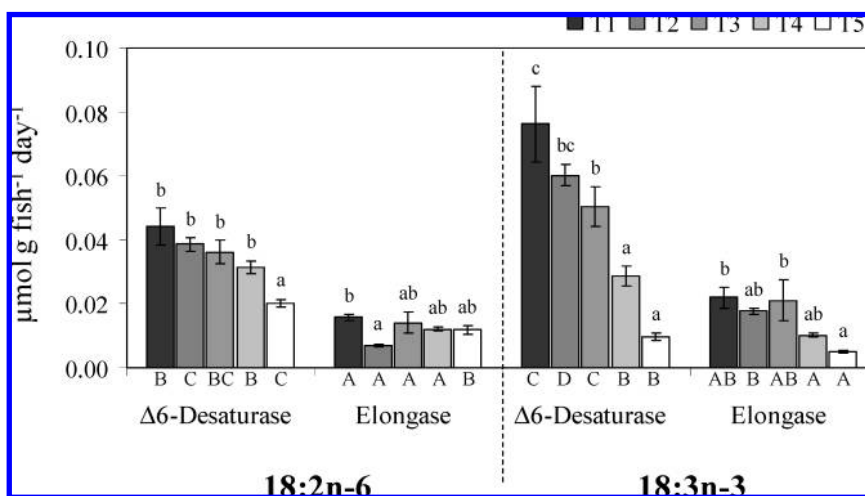


Figure 1. Apparent *in vivo* elongase and Δ -6 desaturase activity on 18:2n-6 and 18:3n-3 (μmol of end products per g of fish per day; mean \pm S.E.M; $N = 3$). On top of the bars, lower case letters that are the same indicate no significant differences between treatments. At the base of the bars, upper case letters that are the same indicate no significant differences within treatments.

Δ -6 desaturase activity acting on 18:2n-6 and 18:3n-3 was plotted against their respective net dietary inclusion and expressed as $\mu\text{mol g}^{-1} \text{day}^{-1}$ or % of net intake (Figure 2). For both fatty acids, a positive linear regression described the relationships between dietary content (substrate availability) and enzyme activity when expressed as $\mu\text{mol g}^{-1} \text{day}^{-1}$, indicating that the higher the substrate availability the higher the enzyme end product. However, when the activity was expressed as % of net intake (% of substrate availability), a negative linear regression was found for 18:2n-6, indicating that the efficiency of the Δ -6 desaturase was proportionally reduced with the increase of substrate availability (Figure 2A). Δ -6 desaturase activity on 18:3n-3 was, on the contrary, described by a second order polynomial equation, indicating an initial increase of enzyme efficiency in parallel with substrate availability and then decreasing after peaking at $1.048 \mu\text{mol g}^{-1} \text{day}^{-1}$ of substrate availability (Figure 2B). Similarly, when total Δ -6 desaturase activity acting on 18:2n-6 and 18:3n-3 is plotted relative to the total combined substrate availability, there is a positive linear correlation when expressed as $\mu\text{mol g}^{-1} \text{day}^{-1}$ and a second order polynomial equation, when expressed as % of substrate availability, peaking when the total net intake of 18:2n-6 and 18:3n-3 was $2 \mu\text{mol g}^{-1} \text{day}^{-1}$ (Figure 3A). The relationships between the percentage of total Δ -6 desaturase activity on 18:2n-6 and 18:3n-3, in relation to total net intake are described in Figure 3B. *In vivo*, it was evident that at low C_{18} fatty acid dietary inclusion (T5), the majority of the Δ -6 desaturase activity acted on 18:2n-6, decreasing to the theoretical lower limit of 33.86% at the high level of C_{18} fatty acid

dietary inclusion (T1), while the opposite trend was apparent for 18:3n-3, peaking to the theoretical maximum of 66.14% of total Δ -6 desaturase acting on 18:3n-3.

DISCUSSION

The experimental diets were intentionally deprived of fishmeal in consideration that its content of n-3 LCPUFA could potentially mask the effects of the fish oil substitution. Consequently, it is not surprising that fish receiving the control fish oil-based diet (CD) demonstrated better growth performance, as previously described in Murray cod (7) and other finfish species (26). Overall, the growth performance of Murray cod was relatively poor in comparison to previously published data for this species (19, 27) and also in experiments in which fish oil was substituted by terrestrial alternatives (7, 18, 20, 23). This could partially be explained by a negative effect of the inclusion of wheat gluten in the diet formulation, which has been reported to have low a phosphorus (28) and lysine content (29). However, a previous investigation on Murray cod showed no negative effect of the inclusion of wheat gluten on performance, although at a somewhat lower inclusion level (30).

The fatty acid makeup of experimental diets differed slightly from what was expected. This was attributable to higher than expected concentrations of 18:2n-6, particularly evident in diets formulated to contain low amounts of C_{18} PUFA. This was likely due to the contribution of the nonlipid sources in the diet, mainly soybean, wheat gluten, flour, and fishmeal which, although limited in their lipid concentrations, commonly contain higher

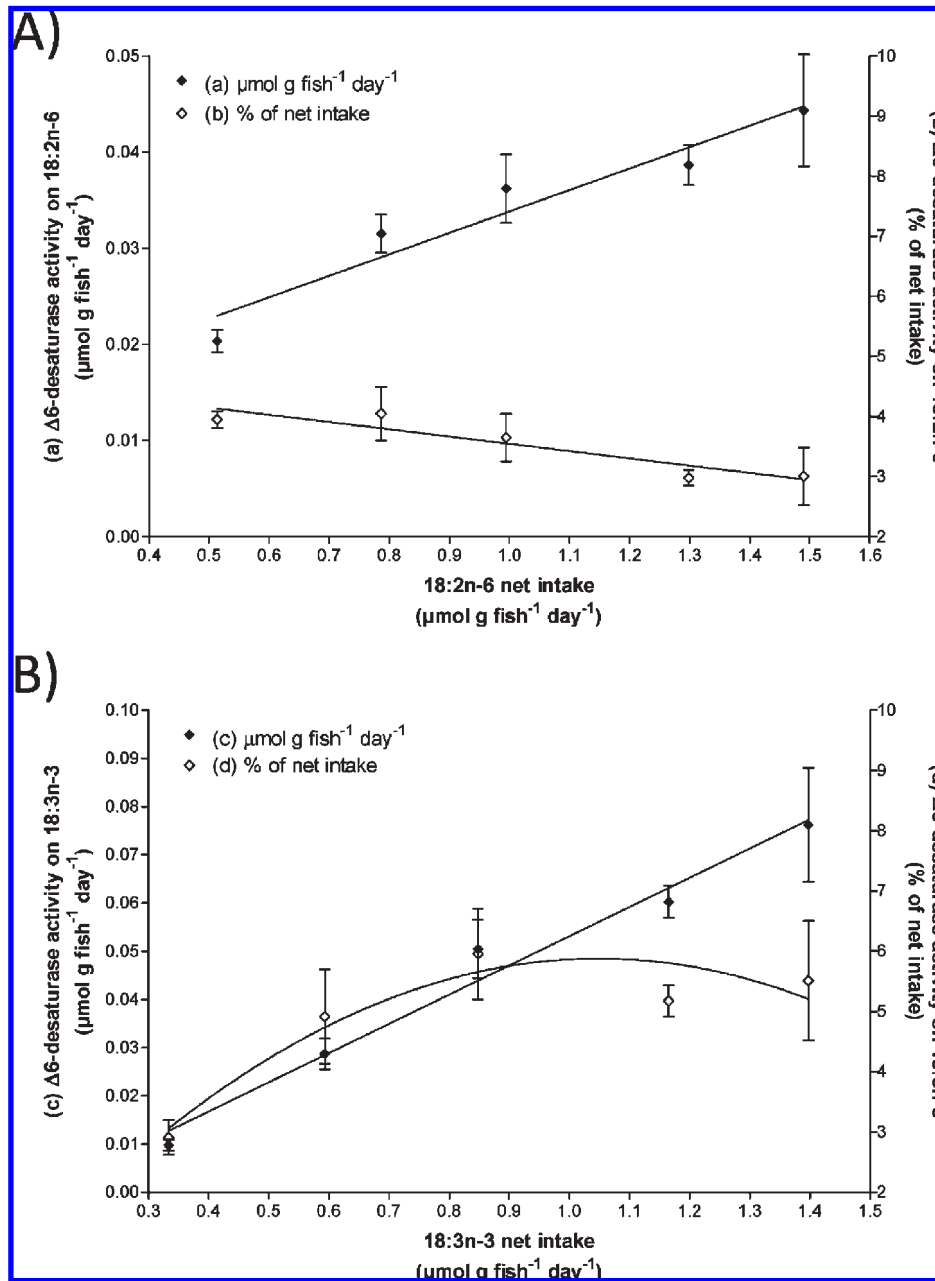


Figure 2. (A) Total apparent in vivo $\Delta 6$ -desaturase activity on 18:2n-6 expressed as (a) $\mu\text{mol g of fish}^{-1} \text{ day}^{-1}$ and as (b) % of net intake, in relationship to the 18:2n-6 net intake ($\mu\text{mol g of fish}^{-1} \text{ day}^{-1}$). Regression equations: (a) $y = 0.02232x + 0.01152$, $R^2 = 0.9302$, $p < 0.05$; (b) $y = -1.208x + 4.753$, $R^2 = 0.8582$, $p < 0.05$. (B) Total apparent in vivo $\Delta 6$ -desaturase activity on 18:3n-3 expressed as (c) $\mu\text{mol g of fish}^{-1} \text{ day}^{-1}$ and as (d) % of net intake, in relationship to the 18:3n-3 net intake ($\mu\text{mol g of fish}^{-1} \text{ day}^{-1}$). Regression equations: (c) $y = 0.0606x - 0.007516$, $R^2 = 0.9778$, $p < 0.05$; (d) $y = -5.516x^2 + 11.57x - 0.1870$, $R^2 = 0.9620$, $p < 0.05$.

levels of 18:2n-6 in comparison to that of 18:3n-3 (31). Consequently, the 18:3n-3/18:2n-6 ratio varied slightly from the anticipated 1/1 ratio. As expected and previously reported, the dietary fatty acid composition was reflected in the fish fatty acid makeup (32, 33). Similarly, the results of the digestibility evaluation were in accordance with previously published studies, in which a preferential order of absorption of PUFA > MUFA > SFA and short chain > longer chain fatty acids was demonstrated (34, 35).

Via the implementation of the whole-body fatty acid balance method, it was possible to estimate the apparent in vivo β -oxidation of individual fatty acids (24). In a previous study, no clear trends were observed in individual fatty acid oxidation in Murray cod fed diets in which fish oil was incrementally replaced by a vegetable oil blend (18). However, previous studies on

Atlantic salmon (*Salmo salar*) have indicated that 18:2n-6, 18:3n-3, and 18:1n-9 are readily β -oxidized when present in high dietary concentrations (8, 36). Moreover, it has also been shown that 20:5n-3 is actively β -oxidized when present in large quantities in the diet (37). The findings of the present study support these previous observations in consideration that 18:2n-6, 18:3n-3, 18:1n-9, and 20:5n-3 were largely β -oxidized in fish receiving diets abundant in these fatty acids. Consequently, despite the theoretical existence of a preferential order of β -oxidation for certain fatty acids over others, these differences are commonly hidden when dietary fatty acids are administered in surplus (25). Interestingly, although the levels of 18:2n-6 in the five experimental diets (T1-T5) were slightly higher than those of 18:3n-3, the level of β -oxidation of 18:3n-3 was greater than that of 18:2n-6 in treatments T1, T2, and T3, likely indicating that 18:3n-3 is the

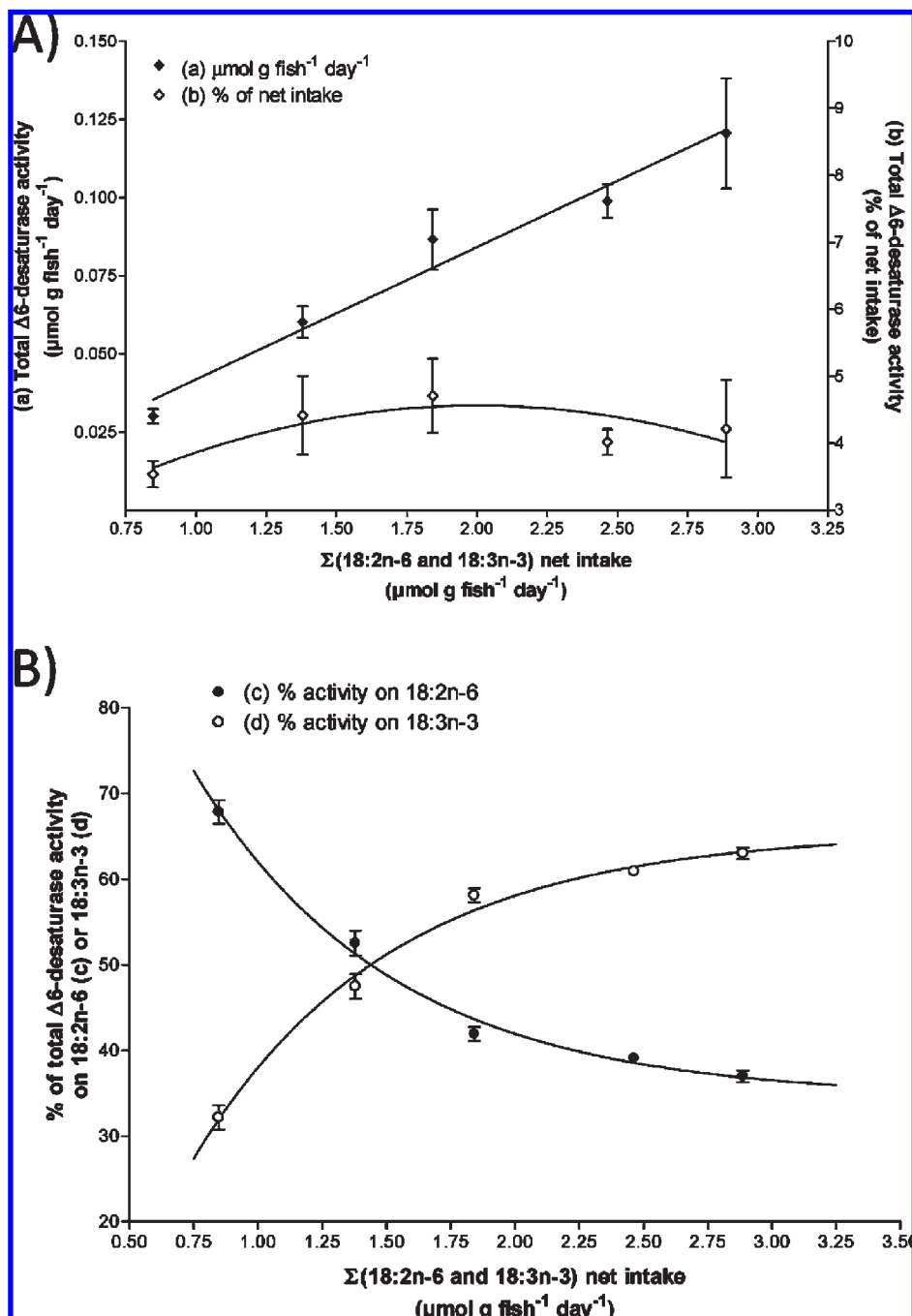


Figure 3. (A) Total apparent in vivo Δ -6 desaturase activity (on 18:2n-6 and 18:3n-3) expressed as (a) $\mu\text{mol g of fish}^{-1} \text{ day}^{-1}$ and as (b) % of net intake, in relationship to the total net intake of 18:2n-6 and 18:3n-3 ($\mu\text{mol g of fish}^{-1} \text{ day}^{-1}$). Regression equations: (a) $y = 0.0423x + 0.004330$, $R^2 = 0.9711$, $p < 0.05$; (b) $y = -0.6971x^2 + 2.791x + 1.791$, $R^2 = 0.8927$, $p < 0.05$. (B) Percentage of total apparent in vivo Δ -6 desaturase activity acting on (c) 18:2n-6 and (d) 18:3n-3 plotted against total in vivo available substrate (the total net intake of 18:2n-6 and 18:3n-3, $\mu\text{mol g of fish}^{-1} \text{ day}^{-1}$). Regression equations: (c) $y = 101.7e^{-1.307x} + 34.50$, $R^2 = 0.9975$; (d) $y = -101.7e^{-1.307x} + 65.50$, $R^2 = 0.9975$.

preferential substrate for β -oxidation when a large amount of dietary C_{18} PUFA is provided.

In the present study, the apparent in vivo 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, and this, as previously reported, is one of the practical limits of the whole-body fatty acid balance method (24). However, interesting trends in the apparent activity of the Δ -6 desaturase were recorded. The total apparent enzyme activity was greater in fish receiving higher amounts of dietary C_{18} PUFA, clearly indicating a direct relationship between enzyme substrate and enzyme product (Figure 1). However, for 18:2n-6, the

efficiency to which the substrate (18:2n-6) was desaturated (18:3n-6) was inversely correlated to substrate availability (Figure 2A), while for 18:3n-3, the maximum efficiency of apparent Δ -6 desaturase activity was recorded for an in vivo substrate availability of $1 \mu\text{mol g}^{-1} \text{ day}^{-1}$ (Figure 2B). Similarly, when the combined activity of the apparent Δ -6 desaturase on 18:2n-6 and 18:3n-3 was considered (Figure 3A), the maximum efficiency was recorded for a diet providing a total substrate availability of $1 \mu\text{mol g}^{-1} \text{ day}^{-1}$. This substrate availability equates to a diet containing approximately 420 mg g^{-1} (or 45%) of C_{18} PUFA with an 18:3n-3/18:2n-6 ratio of 1/1. It is envisaged that this information will prove useful for the

development of low fish oil diets for Murray cod that will permit the maintenance of n-3 LCPUFA levels.

Interestingly, fish receiving higher levels of C₁₈ PUFA had a greater amount of apparent Δ-6 desaturase activity acting on 18:3n-3 than on 18:2n-6. With the gradual reduction of dietary C₁₈ PUFA (T1 to T5), the substrate preference of Δ-6 desaturase between 18:3n-3 and 18:2n-6 shifted (**Figure 1**). This trend was underlined by the relationships between the combined (18:2n-6 and 18:3n-3) substrate availability and the percentage of total apparent Δ-6 desaturase activity acting on 18:2n-6 or on 18:3n-3 (**Figure 3B**). This indicated an increase in Δ-6 desaturase affinity toward the 18:3n-3 substrate rather than 18:2n-6 when C₁₈ PUFA was provided in abundance. This may suggest that the most pressing need for Murray cod is 20:4n-6, above that of 20:5n-3 and 22:6n-3, and hence a higher affinity toward 18:2n-6 when substrate intake is low. Once this requirement has been met and if sufficient substrate is still available, then Δ-6 desaturase exhibits an increased affinity toward the n-3 substrate. The affinity of Δ-6 desaturase activity for fatty acids in the order of n-3 < n-6 has been well-documented in teleost fish species (10) and previously confirmed in Murray cod (16, 18). However, in the majority of studies, an abundance of enzyme substrate (C₁₈ PUFA) was provided in the diet.

Unfortunately, for fish and vertebrates in general, little information is available regarding the modulation of enzyme affinity toward n-3 and n-6 substrates in relation to substrate availability. This paucity of information is likely attributable to the intrinsic technical difficulties associated with the measurement of desaturase activity (38) and the consequent impracticability of conventional enzymological assessment.

The results of this research show that an increase in the in vivo availability of Δ-6 desaturase substrate (i.e., 18:3n-3) results in an increase of enzyme product content (18:4n-3) in fish tissue. However, the maximal efficiency of the enzyme is reached at an average substrate availability, and an excessively high dietary content of C₁₈ PUFA may prove counterproductive when aiming to maximize in vivo n-3 LCPUFA production. Furthermore, Murray cod demonstrated a shift in the in vivo Δ-6 desaturase enzyme affinity from 18:3n-3 to 18:2n-6, when dietary C₁₈ PUFA, the enzyme substrate, was reduced.

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