

Fatty Acid Profile of Sunshine Bass: II. Profile Change Differs Among Fillet Lipid Classes

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Abstract Fatty acid (FA) profile of fish tissue mirrors dietary FA profile and changes in a time-dependent manner following a change in dietary FA composition. To determine whether FA profile change varies among lipid classes, we evaluated the FA composition of fillet cholesteryl esters (CE), phospholipids (PL), and triacylglycerols (TAG) of sunshine bass (SB, *Morone chrysops* × *M. saxatilis*) raised on feeds containing fish oil or 50:50 blend of fish oil and coconut, grapeseed, linseed, or poultry oil, with or without implementation of a finishing period (100% FO feed) prior to harvest. Each lipid class was associated with a generalized FA signature, irrespective of nutritional history: fillet PL was comprised largely of saturated FA (SFA), long-chain polyunsaturated FA (LC-PUFA), and total n-3 FA; fillet TAG was higher in MC-PUFA and total n-6 FA; and fillet CE was highest in monounsaturated FA (MUFA). Neutral lipids reflected dietary composition in a near-direct fashion; conversely, PL showed evidence of selectivity for MC- and LC-PUFA. Shorter-chain SFA were not strongly reflected within any lipid fraction, even when dietary availability was high, suggesting catabolism of these FA. FA metabolism in SB is apparently characterized by a division between saturated and unsaturated FA, whereby LC-PUFA are preferentially incorporated into tissues and SFA are preferentially oxidized for energy production. We demonstrated provision of SFA in grow-out feeds for SB, instead MC-PUFA which compete for tissue deposition, meets energy demands and allows for maximum inclusion of LC-PUFA within fillet lipids.

Keywords Fish oil · Alternative lipid · Finishing feed · Fatty acid turnover/dilution · LC-PUFA · *Morone* spp.

Introduction

Fish oil (FO) replacement has been investigated for a large number of aquaculture species, employing a variety of alternative lipid sources, such as corn, canola, soybean, linseed, and palm oils [1–4]. In general, these studies have demonstrated FO can be partially or completely replaced in aquafeeds without affecting growth performance of the livestock. We previously demonstrated canola, coconut, corn, grapeseed, linseed, and poultry oils are effective substitutes for FO in feeds for sunshine bass (SB, *Morone chrysops* × *M. saxatilis*) [3–5]. Unfortunately, FO replacement significantly alters the fatty acid (FA) composition of the resultant product and results in a corresponding decline in fillet long-chain polyunsaturated fatty acids (carbon atoms ≥ 20 , degree of unsaturation ≥ 3 , LC-PUFA), such as eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3). Fish tissues are plastic with respect to FA composition, and reflect recent nutritional history, that is, tissue FA profile mirrors dietary FA profile and changes in a time-dependent manner following modification of dietary composition [6–9]. Compared to FO, alternative oils are typically higher in monounsaturates (MUFA) and medium-chain polyunsaturated fatty acids (18 carbon, degree of unsaturation ≥ 2 , MC-PUFA), whereas LC-PUFA are low or lacking altogether. Accordingly, fillets of fish fed alternative oil-based feeds reflect these differences: replacing LC-PUFA-rich FO with MC-PUFA-rich canola oil, for example, increases fillet 18:2n-6 and 18:3n-3 content at the expense of 20:5n-3 and 22:6n-3 [3, 4]. Although cultured seafood produced

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using alternative lipids sources are attractive from sustainability and production cost standpoints, they are also substantially less valuable in terms of providing beneficial LC-PUFA to human consumers.

The inherent conflict between production of fillets with the greatest nutritional value (FO-based feeds) and the lowest feed costs (alternative lipid-based feeds) may be overcome through the use of “finishing” feeds to augment fillet LC-PUFA content of fish raised on alternative feeds prior to harvest. As mentioned above, fillet FA composition changes following dietary modification to more closely reflect the profile of the new feed. Finishing strategies exploit this process by implementing high-LC-PUFA content feeds at the end of the production cycle to restore fillet LC-PUFA content immediately prior to harvest. Employing FO-based finishing feeds, fillet LC-PUFA content of fish raised on alternative lipid-based grow-out feeds was augmented [10–18]; however, complete profile restoration has been achieved in very few studies [17, 18]. The process of FA profile change appears to differ among lipid classes [12, 17], leading some to attribute variation in finishing success to differences in fillet lipid content and class composition. We previously assessed production performance and finishing success of SB raised on multiple alternative lipid sources [5]. The purpose of the present work was to determine whether FA profile change varied among fillet lipid classes.

Methods and Materials

In the following sections, we describe our experimental design and provide a brief summary of the pertinent methods. Animal husbandry, sample collection, and analysis are described in detail in our previous paper [5]. All procedures were conducted under the direction and approval of the Southern Illinois University Institutional Animal Care and Use Committee, protocol #07-008.

Experimental Feeds

Five feeds were manufactured based on a practical, reduced fish meal feed our group had previously developed for SB culture [5]. As originally formulated, this feed contained 9.8% FO (dry matter basis; menhaden-derived; Virginia Gold™, Omega Protein, Inc., Houston, TX, USA). In the present work, the original, FO-based formulation served as the control grow-out feed as well as the finishing feed. Four experimental grow-out feeds were derived from the basal formulation, with 50% of the FO replaced with poultry (PO, Tyson Foods, Inc., Robards, KY, USA), linseed (LO, Barlean's Organic Oils, Ferndale, WA, USA), grapeseed (GO, Kusha, Inc, Irvine, CA, USA), or coconut oil (CO,

Spectrum Organic Products, Petaluma, CA, USA). Dietary formulations and FA composition data are provided in Tables 1 and 2.

Experimental Design and Feeding Trial

Nine feeding regimens were developed to address influences of dietary lipid source and FA composition on production performance and subsequent finishing diet success in SB culture. Eight experimental regimens represented feeding the grow-out feeds described above throughout the feeding trial (CO, LO, GO, and PO regimens) or with an 8-week finishing period (CO + Finish, LO + Finish, GO + Finish, and PO + Finish regimens). The control regimen represented feeding the FO control/finishing feed throughout the duration of the feeding trial (FO Control regimen). Juvenile SB [46 ± 1 g, mean ± SE; age 1 (~10 months); Keo, Fish Farm, Keo AR] were fed the grow-out feeds for 20 weeks until they reached a submarketable size (328 ± 16 g). After 20 weeks, subsamples of fish were harvested to determine baseline tissue FA profile prior to finishing. After baseline sampling, remaining fish were finished with the 100% FO feed for the remaining 8 weeks of the feeding trial (CO + Finish, LO + Finish, GO + Finish, and PO + Finish regimens) or maintained on assigned grow-out feeds (FO Control, CO, LO, GO, and PO regimens). After completion of the feeding trial, all remaining fish were harvested. Survival and production performance criteria were assessed, and muscle samples were collected to determine fillet FA composition.

Lipid Class Separations and Fatty Acid Analysis

Total lipid was extracted from lyophilized fillet tissue as previously described [5]. Phospholipid (PL), cholesteryl ester (CE), and triacylglycerol (TAG) fractions were separated via solid-phase extraction according to Burdge et al. [19]. Briefly, ~75–100 mg of total lipid was suspended in chloroform and applied to amino-propyl silica columns (Supelclean™ LC-NH2 1 mL SPE columns, Supelco, Bellefonte, PA, USA) under gravity. The columns were washed with 2 mL of chloroform under a vacuum to elute a mixed TAG and CE fraction. The column was subsequently washed with 1 mL each of chloroform:methanol solution (3:2 v/v) and methanol to elute the phospholipid fraction. The mixed TAG and CE fraction was dried under nitrogen, resuspended in hexane, and applied to a fresh column (preconditioned with hexane). The column was washed with 2 mL of hexane followed by 2 mL of hexane:chloroform:ethyl acetate solution (100:5:5 v/v) to elute the CE and TAG fractions, respectively. Burdge et al. [19] also allows for separation of free fatty acids; however,

Table 1 Formulation and proximate composition of experimental feeds

Ingredient	Fish oil (FO)	Coconut oil (CO)	Grapeseed oil (GO)	Linseed oil (LO)	Poultry oil (PO)
Fish meal ^a	200	200	200	200	200
Fish oil ^a	98	49	49	49	49
Coconut oil ^b	–	49	–	–	–
Grapeseed oil ^c	–	–	49	–	–
Linseed oil ^d	–	–	–	49	–
Poultry oil ^e	–	–	–	–	49
Corn Gluten meal ^f	140	140	140	140	140
Wheat middlings	201	201	201	201	201
Soybean meal	300	300	300	300	300
Carboxymethyl cellulose	20	20	20	20	20
Sodium phosphate monobasic	15	15	15	15	15
Calcium phosphate Dibasic	15	15	15	15	15
Choline chloride	6	6	6	6	6
Mineral premix ^g	1.5	1.5	1.5	1.5	1.5
Vitamin premix ^h	1.5	1.5	1.5	1.5	1.5
Proximate composition					
Dry matter	88.0	87.8	88.3	84.7	85.7
Protein	41.1	41.5	41.0	40.5	39.4
Lipid	14.3	14.5	14.4	14.6	13.2
Ash	10.8	10.2	9.6	11.4	10.6

All proximate composition values are expressed as a mean in g/100g(%), dry matter basis. Formulation composition is expressed in g/kg

^a Derived from menhaden *Brevoortia* spp., Omega Protein, Inc., Houston, TX, USA

^b Spectrum Organic Products, Petaluma, CA, USA

^c Kusha, Inc., Irvine, CA, USA

^d Barlean's Organic Oils, Ferndale, WA, USA

^e Tyson Foods, Inc., Robards, KY, USA

^f Tate and Lyle, Decatur, IL, USA

^g Formulated to contain: 7,000 mg kg⁻¹ copper, 70,000 mg kg⁻¹ iron, 100,000 mg kg⁻¹ manganese, 200,000 mg kg⁻¹ zinc, 0.24% iodine

^h Formulated to contain: 99.8 mg kg⁻¹ selenium, 2,200 mg kg⁻¹ folic acid, 88,000 mg kg⁻¹ niacin, 35,200 mg kg⁻¹ pantothenic acid, 11,000 mg kg⁻¹ vitamin B6, 13,200 mg kg⁻¹ riboflavin, 11,000 mg kg⁻¹ thiamin, 11,000 mg kg⁻¹ vitamin B12, 66,000 mg kg⁻¹ vitamin E, 4,400 mg kg⁻¹ vitamin K, 4,400,000 IU kg⁻¹ vitamin A, 2,200,000 IU kg⁻¹ vitamin D, 100,000 mg kg⁻¹ vitamin C

amounts recovered from fillet total lipid were extremely small (0.1–0.2 mg) and insufficient for subsequent FA analysis. Once separated, each lipid class was evaporated to dryness under nitrogen and weighed to determine total tissue PL, CE, and TAG content. All lipid samples were then resuspended in toluene, subjected to acid-catalyzed trans-methylation, and analyzed for FA composition as described in our previous paper [5].

Statistical Analysis

Although multiple individual fish were sampled from each tank, replicate tanks served as the experimental units for all statistical analyses ($n = 3$ for experimental regimens, $n = 6$ for control regimen). Lipid class data were analyzed by one-way analysis of variance (ANOVA) within the Mixed Model framework of the Statistical Analysis System,

version 9.1 (SAS Institute, Cary, NC, USA) to determine significance of differences among feeding regimens. In all cases, differences were considered significant at $P < 0.05$.

Results

Across all lipid classes, FA profiles of unfinished groups generally reflected the FA composition of the grow-out feed (Tables 3, 4, 5). Increased levels of 12:0, 18:1n-9, 18:3n-3, and 18:2n-6 were associated with all lipid classes of the CO, PO, LO, and GO groups, respectively; however, the extent to which the tissue reflected the diet varied among individual lipid classes. Among unfinished groups, the TAG and CE fractions showed a strong correlation with dietary composition; whereas the PL fraction was less representative of dietary composition (Fig. 1). Comparing

Table 2 Dietary composition with respect to FA and FA classes

Fatty acid(s)	Fish oil (FO)	Coconut oil (CO)	Grapeseed oil (GO)	Linseed oil (LO)	Poultry oil (PO)	SEM
8:0	0.00	1.83	0.00	0.00	0.00	0.05
10:0	0.00	2.02	0.00	0.00	0.00	0.02
12:0	0.11	17.59	0.02	0.04	0.06	0.02
14:0	7.65	11.29	4.41	4.42	4.68	0.02
16:0	18.71	15.12	14.47	13.45	20.87	0.02
18:0	3.50	3.28	3.58	3.71	4.48	0.01
Total SFA ¹	31.90	52.38	23.90	22.97	31.40	0.06
16:1n-7	9.84	5.65	5.62	5.61	7.85	0.02
18:1n-7	2.90	1.75	1.98	1.92	2.45	0.00
18:1n-9	7.74	8.12	13.62	11.16	19.67	0.02
Total MUFA ²	21.68	16.20	21.98	19.40	30.76	0.03
18:2n-6	8.42	8.85	32.40	14.68	15.58	0.08
20:4n-6	0.92	0.58	0.58	0.58	0.71	0.01
n-6	10.12	10.21	33.45	16.02	16.92	0.08
18:3n-3	2.14	2.04	1.59	22.56	1.82	0.01
18:4n-3	3.49	1.86	1.86	1.87	1.82	0.01
20:4n-3	1.49	0.81	0.84	0.81	0.81	0.01
20:5n-3	11.46	6.60	6.53	6.53	6.52	0.02
22:5n-3	2.11	1.22	1.28	1.22	1.21	0.02
22:6n-3	12.73	6.96	6.86	6.89	7.04	0.03
n-3	33.64	19.60	19.08	40.02	19.34	0.05
Total PUFA ³	46.42	31.42	54.12	57.63	37.84	0.07
Total LC-PUFA ⁴	29.13	16.40	16.33	16.30	16.59	0.05
Total MC-PUFA ⁵	14.72	13.16	36.26	39.53	19.69	0.08
n-3:n-6	3.32	1.92	0.57	2.50	1.14	0.02

Values represent least-square means of triplicate samples

¹ Saturated fatty acids—sum of all FA without double bonds

² Monounsaturated fatty acids—sum of all FA with a single double bond

³ Polyunsaturated fatty acids—sum of all FA with ≥ 2 double bonds

⁴ Long-chain PUFA—sum of all FA with chain length ≥ 20 carbon atoms and double bonds ≥ 3

⁵ Medium-chain—sum of all PUFA with chain length of 18 carbon atoms; includes 18:3n-4 in addition to individually reported MC-PUFA

the CO regimen with the FO Control regimen, the difference between 12:0 levels was greatest within the neutral TAG and CE fractions (1.1 vs. 9.5% for TAG, 1.0 vs. 8.6% for CE Tables 4 and 5) and minor, though statistically significant within the polar PL fraction (0.0 vs. 0.5%; Table 3). Differences in 18:1n-9 content between the FO Control and PO regimens were similarly partitioned among the neutral and polar lipid classes, with differences of the greatest magnitude observed within the TAG and CE. Although 18:3n-3 and 18:2n-6 enrichment within the LO and GO regimens was also greatest within the neutral lipid, substantial increases in these FA were also observed within the PL fraction.

Implementation of the 8-week finishing period reversed grow-out related changes in fillet FA composition to varying degrees (Tables 3, 4, 5). Absolute levels of LC-PUFA were increased in all lipid classes by finishing;

however, differences between finished and unfinished groups were not statistically significant in all cases, particularly among the PL fraction (Tables 3, 4, 5). Among finished groups, increased levels of LC-PUFA were accompanied by decreased levels of MUFA and MC-PUFA. Reduced levels of MUFA and MC-PUFA in finished groups compared to unfinished groups were most noticeable among the PL fraction (Table 3).

Each lipid class was associated with a generalized FA signature, irrespective of nutritional history, which indicated differences in partitioning of dietary FA within the lipid classes. The PL fraction represented $\sim 14\%$ of the total fillet lipid (Table 3) and was comprised largely of SFA (specifically 16:0 and 18:0), LC-PUFA (specifically 20:4n-6, 20:5n-3, and 22:6n-3), and n-3 FA (Table 6). Conversely, the TAG fraction ($\sim 10\%$ of total lipid, Table 5) contained greater amounts of MC-PUFA

Table 3 Fillet phospholipid fatty acid composition with respect to predominant (>1% fatty acid methyl esters, FAME) fatty acid (FA) and FA classes

Fatty acid(s)	Fish (FO)		Coconut (CO)		Grapeseed (GO)		Linseed (LO)		Poultry (PO)	
	FO Control	CO	CO + Finish	GO	GO + Finish	LO	LO + Finish	PO	PO + Finish	
12:0	0.0 ± 0.0 ^b	0.5 ± 0.1 ^a	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	
14:0	1.7 ± 0.0 ^b	3.5 ± 0.1 ^a	1.9 ± 0.1 ^b	1.2 ± 0.0 ^c	1.6 ± 0.0 ^b	1.2 ± 0.0 ^c	1.7 ± 0.1 ^b	1.0 ± 0.1 ^c	1.6 ± 0.0 ^b	
16:0	30.2 ± 0.5 ^a	28.6 ± 0.3 ^{abc}	30.2 ± 0.4 ^a	27.4 ± 0.8 ^b	29.4 ± 0.5 ^{abc}	27.0 ± 0.4 ^c	30.0 ± 0.7 ^{ab}	29.3 ± 0.3 ^{abc}	29.0 ± 0.2 ^{abc}	
18:0	4.0 ± 0.1	4.1 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	4.1 ± 0.0	4.1 ± 0.1	3.7 ± 0.1	3.9 ± 0.1	
SFA ¹	36.6 ± 0.6 ^a	37.4 ± 0.4 ^a	36.8 ± 0.4 ^a	33.3 ± 0.9 ^{bc}	35.7 ± 0.5 ^{abc}	33.0 ± 0.4 ^c	36.6 ± 0.8 ^{ab}	34.6 ± 0.3 ^{abc}	35.3 ± 0.2 ^{abc}	
16:1n-7	2.4 ± 0.2 ^a	7.4 ± 0.2 ^{ab}	2.4 ± 0.1 ^a	1.5 ± 0.0 ^c	2.3 ± 0.1 ^a	1.6 ± 0.0 ^c	2.4 ± 0.1 ^a	1.9 ± 0.1 ^{bc}	2.4 ± 0.1 ^a	
18:1n-7	2.4 ± 0.1 ^a	1.8 ± 0.1 ^{bc}	2.2 ± 0.1 ^{abc}	2.0 ± 0.1 ^{abc}	2.4 ± 0.1 ^a	1.7 ± 0.1 ^c	2.3 ± 0.1 ^{ab}	1.9 ± 0.1 ^{bc}	2.2 ± 0.1 ^{ab}	
18:1n-9	7.8 ± 0.4 ^c	8.7 ± 0.3 ^{bc}	7.8 ± 0.3 ^c	9.4 ± 0.4 ^b	8.1 ± 0.2 ^{bc}	9.4 ± 0.1 ^b	8.6 ± 0.2 ^{bc}	11.2 ± 0.3 ^a	8.4 ± 0.1 ^{bc}	
MUFA ²	12.7 ± 0.2 ^b	12.9 ± 0.4 ^b	12.6 ± 0.3 ^b	13.1 ± 0.5 ^b	13.0 ± 0.4 ^b	12.9 ± 0.1 ^b	13.7 ± 0.2 ^{ab}	15.2 ± 0.4 ^a	13.2 ± 0.3 ^b	
18:2n-6	4.2 ± 0.0 ^c	6.0 ± 0.1 ^d	4.6 ± 0.1 ^c	18.3 ± 0.3 ^a	7.4 ± 0.2 ^c	9.4 ± 0.1 ^b	6.2 ± 0.3 ^d	9.5 ± 0.3 ^b	5.6 ± 0.1 ^d	
20:4n-6	3.0 ± 0.0 ^a	2.6 ± 0.0 ^{ab}	3.1 ± 0.2 ^a	2.3 ± 0.1 ^b	2.9 ± 0.1 ^a	2.6 ± 0.1 ^{ab}	2.9 ± 0.1 ^a	3.0 ± 0.1 ^a	3.1 ± 0.1 ^a	
n-3 ³	7.8 ± 0.1 ^g	9.5 ± 0.1 ^e	8.4 ± 0.2 ^{fg}	21.9 ± 0.3 ^a	11.1 ± 0.1 ^d	12.8 ± 0.2 ^c	9.8 ± 0.3 ^e	13.3 ± 0.3 ^b	9.3 ± 0.1 ^{ef}	
18:3n-3	0.4 ± 0.0 ^c	0.5 ± 0.0 ^c	0.4 ± 0.0 ^c	0.5 ± 0.0 ^c	0.4 ± 0.0 ^c	6.2 ± 0.1 ^a	1.8 ± 0.2 ^b	0.6 ± 0.0 ^c	0.4 ± 0.0 ^c	
18:4n-3	0.3 ± 0.0 ^{ab}	0.3 ± 0.0 ^a	0.3 ± 0.0 ^a	0.2 ± 0.0 ^d	0.2 ± 0.0 ^{abc}	0.2 ± 0.0 ^{bcd}	0.3 ± 0.0 ^a	0.2 ± 0.0 ^{cd}	0.3 ± 0.0 ^a	
20:5n-3	14.9 ± 0.3 ^a	15.6 ± 0.4 ^a	15.1 ± 0.3 ^a	9.9 ± 0.5 ^b	14.2 ± 0.3 ^a	11.6 ± 0.1 ^b	14.4 ± 0.6 ^a	12.8 ± 0.2 ^b	15.1 ± 0.3 ^a	
22:5n-3	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.3	1.6 ± 0.0	1.8 ± 0.1	
22:6n-3	24.9 ± 0.6 ^a	21.5 ± 0.8 ^{ab}	24.0 ± 0.9 ^a	19.2 ± 1.0 ^b	23.0 ± 0.9 ^{ab}	21.2 ± 0.3 ^{ab}	21.5 ± 0.8 ^{ab}	21.3 ± 0.9 ^{ab}	23.9 ± 0.3 ^{ab}	
n-3 ⁴	42.5 ± 0.7 ^a	40.0 ± 0.8 ^{ab}	41.9 ± 0.9 ^{ab}	31.5 ± 1.5 ^c	40.0 ± 0.7 ^{ab}	41.1 ± 0.3 ^{ab}	39.8 ± 1.2 ^{ab}	36.8 ± 0.9 ^b	41.9 ± 0.3 ^{ab}	
PUFA ⁵	50.5 ± 0.7	49.7 ± 0.8	50.5 ± 0.7	53.6 ± 1.3	51.2 ± 0.8	54.1 ± 0.3	49.9 ± 0.9	50.2 ± 0.7	51.5 ± 0.3	
LC-PUFA ⁶	45.0 ± 0.7 ^a	42.0 ± 0.9 ^{abc}	44.4 ± 0.8 ^{ab}	33.3 ± 1.5 ^d	42.3 ± 0.7 ^{abc}	37.5 ± 0.4 ^{cd}	40.9 ± 1.3 ^{abc}	39.2 ± 1.0 ^{bc}	44.5 ± 0.3 ^{ab}	
MC-PUFA ⁷	5.1 ± 0.0 ^g	7.1 ± 0.1 ^{de}	5.6 ± 0.1 ^{fg}	19.1 ± 0.4 ^a	8.3 ± 0.2 ^d	15.9 ± 0.2 ^b	8.4 ± 0.4 ^d	10.5 ± 0.3 ^c	6.5 ± 0.1 ^{ef}	
n-3:n-6	5.5 ± 0.1 ^a	4.2 ± 0.1 ^{cd}	5.0 ± 0.2 ^{ab}	1.4 ± 0.1 ^g	3.6 ± 0.1 ^{de}	3.2 ± 0.1 ^{ef}	4.1 ± 0.2 ^{cd}	2.8 ± 0.1 ^f	4.5 ± 0.1 ^{bc}	
Total phospholipids (% of total lipid)	14.1 ± 1.4	14.7 ± 2.0	14.8 ± 2.0	14.2 ± 2.0	12.6 ± 2.0	13.9 ± 2.0	13.3 ± 2.0	11.0 ± 2.0	16.2.1 ± 2.0	

Values represent least-square means (relative area % of FAME) ± SE of two individual samples from all tanks. Means within fatty acid(s) with common letter superscripts are not significantly different. Absence of superscripts indicates lack of statistical significance

¹ Saturated fatty acids—sum of all FA without double bonds; includes 15:0 and 17:0 in addition to individually reported SFA

² Monounsaturated fatty acids—sum of all FA with single double bond; includes 20:1n-9 in addition to individually reported MUFA

³ Includes 20:2n-6 and 20:3n-6 in addition to individually reported n-6 FA

⁴ Includes 20:3n-3 in addition to individually reported n-3 FA

⁵ Polyunsaturated fatty acids—sum of all FA with double bonds ≥2; includes 18:3n-4, 20:3n-4, 20:3n-3, 20:4n-3, 20:2n-6, and 20:3n-6 in addition to individually reported PUFA

⁶ Long-chain PUFA—sum of all PUFA with chain length ≥20 carbon atoms, double bonds ≥3; includes 20:3n-3, 20:4n-3, and 20:3n-6 in addition to individually reported LC-PUFA

⁷ Medium chain PUFA—sum of all PUFA with chain length of 18 carbon atoms; includes 18:3n-4 in addition to individually reported MC-PUFA

Table 4 Fillet cholesteryl ester fatty acid composition with respect to predominant (>1% fatty acid methyl esters, FAME) fatty acid (FA) and FA classes

Fatty acid(s)	Fish (FO)		Coconut (CO)		Grapeseed (GO)		Linseed (LO)		Poultry (PO)	
	FO Control		CO		GO		LO		PO	
	CO + Finish	GO	CO + Finish	GO	CO + Finish	GO	LO + Finish	LO	PO + Finish	PO
12:0	1.0 ± 0.1 ^c	8.6 ± 0.5 ^a	3.3 ± 0.2 ^b	0.2 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.0 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d
14:0	6.3 ± 0.1 ^b	9.5 ± 0.3 ^a	7.1 ± 0.2 ^b	3.8 ± 0.1 ^e	3.8 ± 0.2 ^{de}	3.8 ± 0.2 ^{de}	4.7 ± 0.1 ^{cd}	4.0 ± 0.1 ^{de}	4.0 ± 0.1 ^{de}	4.0 ± 0.1 ^{de}
16:0	20.0 ± 0.1 ^a	18.7 ± 0.3 ^{bc}	19.7 ± 0.3 ^{ab}	16.7 ± 0.2 ^d	16.6 ± 0.4 ^d	16.6 ± 0.4 ^d	17.8 ± 0.3 ^c	20.4 ± 0.1 ^a	20.4 ± 0.1 ^a	20.2 ± 0.3 ^a
18:0	3.5 ± 0.1	3.5 ± 0.3	3.7 ± 0.2	3.5 ± 0.1	3.7 ± 0.3	3.7 ± 0.3	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.7 ± 0.3
SFA ¹	31.9 ± 0.2 ^c	41.1 ± 0.3 ^a	34.7 ± 0.2 ^b	24.9 ± 0.2 ^g	25.0 ± 0.5 ^g	25.0 ± 0.5 ^g	27.0 ± 0.4 ^f	28.8 ± 0.1 ^e	28.8 ± 0.1 ^e	30.3 ± 0.3 ^d
16:1n-7	10.4 ± 0.2 ^a	7.9 ± 0.3 ^d	9.1 ± 0.3 ^{bc}	6.3 ± 0.1 ^e	6.6 ± 0.3 ^e	6.6 ± 0.3 ^e	8.4 ± 0.2 ^{cd}	9.2 ± 0.2 ^{bc}	9.2 ± 0.2 ^{bc}	9.8 ± 0.3 ^{ab}
18:1n-7	3.3 ± 0.0 ^a	2.5 ± 0.0 ^d	3.0 ± 0.0 ^{bc}	2.4 ± 0.0 ^d	2.4 ± 0.0 ^d	2.4 ± 0.0 ^d	2.9 ± 0.1 ^c	2.8 ± 0.0 ^c	2.8 ± 0.0 ^c	3.2 ± 0.0 ^{ab}
18:1n-9	12.4 ± 0.2 ^d	12.7 ± 0.3 ^d	12.2 ± 0.3 ^d	15.7 ± 0.3 ^b	14.8 ± 0.3 ^{bc}	14.8 ± 0.3 ^{bc}	13.3 ± 0.2 ^{cd}	22.4 ± 0.5 ^a	22.4 ± 0.5 ^a	16.0 ± 0.9 ^b
MUFA ²	26.9 ± 0.4 ^c	23.8 ± 0.6 ^d	25.1 ± 0.5 ^{cd}	25.1 ± 0.5 ^{cd}	24.5 ± 0.6 ^{cd}	24.5 ± 0.6 ^{cd}	25.3 ± 0.2 ^{cd}	35.4 ± 0.7 ^a	35.4 ± 0.7 ^a	29.9 ± 1.0 ^b
18:2n-6	8.9 ± 0.2 ^e	8.8 ± 0.1 ^e	8.7 ± 0.1 ^e	27.8 ± 0.4 ^a	18.4 ± 0.6 ^b	18.4 ± 0.6 ^b	11.4 ± 0.4 ^d	14.3 ± 0.1 ^c	14.3 ± 0.1 ^c	10.8 ± 0.4 ^d
20:4n-6	1.3 ± 0.1 ^a	1.1 ± 0.1 ^{ab}	1.4 ± 0.1 ^a	0.9 ± 0.0 ^b	1.1 ± 0.1 ^{ab}	1.1 ± 0.1 ^{ab}	1.1 ± 0.0 ^{ab}	1.0 ± 0.1 ^b	1.0 ± 0.1 ^b	1.2 ± 0.1 ^{ab}
n-6 ³	11.0 ± 0.2 ^c	10.7 ± 0.1 ^e	10.9 ± 0.1 ^e	30.1 ± 0.4 ^a	20.6 ± 0.6 ^b	20.6 ± 0.6 ^b	13.5 ± 0.3 ^d	16.1 ± 0.1 ^c	16.1 ± 0.1 ^c	12.9 ± 0.4 ^d
18:3n-3	1.5 ± 0.0 ^c	1.4 ± 0.1 ^c	1.5 ± 0.1 ^c	1.3 ± 0.0 ^c	1.4 ± 0.0 ^c	1.4 ± 0.0 ^c	9.4 ± 0.7 ^b	1.6 ± 0.0 ^c	1.6 ± 0.0 ^c	1.4 ± 0.1 ^c
18:4n-3	1.4 ± 0.1 ^a	1.1 ± 0.1 ^{bc}	1.2 ± 0.1 ^{ab}	0.8 ± 0.0 ^c	1.2 ± 0.0 ^{ab}	1.2 ± 0.0 ^{ab}	1.2 ± 0.1 ^{ab}	1.0 ± 0.0 ^{bc}	1.0 ± 0.0 ^{bc}	1.3 ± 0.1 ^{ab}
20:5n-3	9.3 ± 0.1 ^a	7.6 ± 0.3 ^b	9.1 ± 0.2 ^a	5.6 ± 0.1 ^c	8.2 ± 0.2 ^{ab}	8.2 ± 0.2 ^{ab}	8.1 ± 0.4 ^{ab}	5.8 ± 0.2 ^c	5.8 ± 0.2 ^c	8.6 ± 0.3 ^{ab}
22:5n-3	2.2 ± 0.0 ^a	1.8 ± 0.1 ^{cd}	2.2 ± 0.1 ^{ab}	1.5 ± 0.0 ^{de}	2.0 ± 0.1 ^{bc}	2.0 ± 0.1 ^{bc}	2.0 ± 0.1 ^{ab}	1.4 ± 0.0 ^e	1.4 ± 0.0 ^e	2.0 ± 0.1 ^{abc}
22:6n-3	14.3 ± 0.6 ^a	11.3 ± 0.8 ^{bcd}	14.0 ± 0.6 ^{ab}	9.8 ± 0.6 ^{de}	11.7 ± 0.7 ^{bc}	11.7 ± 0.7 ^{bc}	12.2 ± 0.3 ^{abc}	8.7 ± 0.5 ^e	8.7 ± 0.5 ^e	12.4 ± 0.8 ^{abc}
n-3 ⁴	29.8 ± 0.5 ^b	24.1 ± 0.9 ^d	29.0 ± 0.5 ^{bc}	19.8 ± 0.6 ^e	25.4 ± 0.8 ^d	25.4 ± 0.8 ^d	33.9 ± 0.2 ^a	19.5 ± 0.8 ^e	19.5 ± 0.8 ^e	26.7 ± 1.1 ^{cd}
PUFA ⁵	41.1 ± 0.4 ^c	35.0 ± 0.9 ^d	40.2 ± 0.5 ^c	50.1 ± 0.3 ^a	46.3 ± 0.6 ^b	46.3 ± 0.6 ^b	47.7 ± 0.4 ^{ab}	35.8 ± 0.8 ^d	35.8 ± 0.8 ^d	39.9 ± 0.8 ^c
LC-PUFA ⁶	28.4 ± 0.6 ^a	22.8 ± 1.1 ^{cd}	27.8 ± 0.7 ^{ab}	18.7 ± 0.7 ^{de}	24.1 ± 0.9 ^{bc}	24.1 ± 0.9 ^{bc}	24.6 ± 0.7 ^{bc}	18.0 ± 0.8 ^e	18.0 ± 0.8 ^e	25.4 ± 1.1 ^{abc}
MC-PUFA ⁷	12.1 ± 0.3 ^d	11.6 ± 0.2 ^d	11.3 ± 0.6 ^d	30.1 ± 0.5 ^a	21.3 ± 0.6 ^b	21.3 ± 0.6 ^b	22.3 ± 1.0 ^b	17.0 ± 0.2 ^c	17.0 ± 0.2 ^c	13.8 ± 0.4 ^d
n-3n-6	2.7 ± 0.1 ^a	2.2 ± 0.1 ^{bc}	2.7 ± 0.1 ^{ab}	0.7 ± 0.0 ^e	1.2 ± 0.1 ^d	1.2 ± 0.1 ^d	2.5 ± 0.1 ^{abc}	1.2 ± 0.1 ^d	1.2 ± 0.1 ^d	2.1 ± 0.2 ^c
Total cholesteryl esters (% of total lipid) ⁸	65.1 ± 3.6	70.7 ± 5.0	69.8 ± 5.0	71.3 ± 5.0	70.5 ± 5.0	70.5 ± 5.0	68.9 ± 5.0	68.6 ± 5.0	68.6 ± 5.0	72.2 ± 5.0

Values represent least-square means (relative area % of FAME) ± SE of two individual samples from all tanks. Means within fatty acid(s) with common letter superscripts are not significantly different. Absence of superscripts indicates lack of statistical significance

1–7 All superscripts denote information provided as footnotes in Table 3

8 The distribution of total fillet lipid among the lipid classes was somewhat surprising, specifically the high level of CE relative to PL and TAG. Previous research with SB has indicated CE contributes very little to total fillet lipid (<5% of total lipid) [37, 38]. However, each of these studies employed much smaller, juvenile fish (~30 g final weight), whose tissue composition is likely to differ from that of large (~500 g) fish nearing sexual maturity (well-developed gonadal tissue was observed in many of our fish at harvest). In this respect, one might expect the tissue composition of larger fish to be skewed toward increased levels of storage lipid, i.e., CE and TAG. Additionally, the previous studies by Gatlin and colleagues employed TLC-FID to quantify lipid classes, which may have underestimated CE content. Recovery of PL and TAG fractions with SPE methods is apparently equivalent to TLC; however, recovery of CE is significantly greater using SPE (19)

Table 5 Fillet triacylglycerol fatty acid composition with respect to predominant (>1% fatty acid methyl esters, FAME) fatty acid (FA) and FA classes

Fatty acid(s)	Fish (FO)		Coconut (CO)		Grapeseed (GO)		Linseed (LO)		Poultry (PO)									
	FO Control		CO		CO + Finish		GO		GO + Finish		LO		LO + Finish		PO		PO + Finish	
12:0	1.1 ± 0.1 ^c	9.5 ± 0.3 ^a	3.6 ± 0.1 ^b	0.2 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d	0.1 ± 0.0 ^d
14:0	6.6 ± 0.1 ^c	10.0 ± 0.1 ^a	7.7 ± 0.1 ^b	4.0 ± 0.1 ^e	5.2 ± 0.1 ^d	5.2 ± 0.1 ^d	3.8 ± 0.1 ^e	3.8 ± 0.1 ^e	4.9 ± 0.2 ^{de}	4.3 ± 0.1 ^e	4.3 ± 0.1 ^e	4.3 ± 0.1 ^e	4.3 ± 0.1 ^e	4.3 ± 0.1 ^e	4.3 ± 0.1 ^e	4.3 ± 0.1 ^e	4.3 ± 0.1 ^e	4.3 ± 0.1 ^e
16:0	15.5 ± 0.2 ^{abc}	14.5 ± 0.2 ^{cde}	15.4 ± 0.2 ^{abcd}	12.9 ± 0.1 ^{ef}	14.7 ± 0.2 ^{abcd}	14.7 ± 0.2 ^{abcd}	12.1 ± 0.1 ^f	12.1 ± 0.1 ^f	13.8 ± 0.6 ^{def}	17.4 ± 0.3 ^a	17.4 ± 0.3 ^a	17.4 ± 0.3 ^a	17.4 ± 0.3 ^a	17.4 ± 0.3 ^a	17.4 ± 0.3 ^a	17.4 ± 0.3 ^a	17.4 ± 0.3 ^a	17.4 ± 0.3 ^a
18:0	1.7 ± 0.0 ^e	1.6 ± 0.0 ^e	1.8 ± 0.1 ^c	1.9 ± 0.0 ^{bc}	1.9 ± 0.1 ^{bc}	1.9 ± 0.1 ^{bc}	1.9 ± 0.0 ^{bc}	1.9 ± 0.0 ^{bc}	1.8 ± 0.1 ^{bc}	2.3 ± 0.1 ^a	2.3 ± 0.1 ^a	2.3 ± 0.1 ^a	2.3 ± 0.1 ^a	2.3 ± 0.1 ^a	2.3 ± 0.1 ^a	2.3 ± 0.1 ^a	2.3 ± 0.1 ^a	2.3 ± 0.1 ^a
SFA ¹	25.8 ± 0.3 ^c	36.5 ± 0.4 ^a	29.4 ± 0.2 ^b	19.5 ± 0.2 ^{fg}	22.8 ± 0.2 ^{de}	22.8 ± 0.2 ^{de}	19.5 ± 0.2 ^{fg}	18.5 ± 0.2 ^g	21.4 ± 0.7 ^{ef}	24.8 ± 0.5 ^{cd}	24.8 ± 0.5 ^{cd}	24.8 ± 0.5 ^{cd}	24.8 ± 0.5 ^{cd}	24.8 ± 0.5 ^{cd}	24.8 ± 0.5 ^{cd}	24.8 ± 0.5 ^{cd}	24.8 ± 0.5 ^{cd}	24.8 ± 0.5 ^{cd}
16:1n-7	11.1 ± 0.1 ^a	8.9 ± 0.0 ^c	10.5 ± 0.1 ^{ab}	7.0 ± 0.1 ^d	9.3 ± 0.1 ^c	9.3 ± 0.1 ^c	7.0 ± 0.1 ^d	6.8 ± 0.1 ^d	9.0 ± 0.3 ^c	10.1 ± 0.1 ^b	10.1 ± 0.1 ^b	10.1 ± 0.1 ^b	10.1 ± 0.1 ^b	10.1 ± 0.1 ^b	10.1 ± 0.1 ^b	10.1 ± 0.1 ^b	10.1 ± 0.1 ^b	10.1 ± 0.1 ^b
18:1n-7	2.9 ± 0.0 ^a	2.3 ± 0.1 ^{cd}	2.7 ± 0.0 ^{cd}	2.1 ± 0.0 ^b	2.5 ± 0.0 ^c	2.5 ± 0.0 ^c	2.1 ± 0.0 ^b	2.1 ± 0.0 ^c	2.5 ± 0.1 ^{cd}	2.7 ± 0.0 ^{ab}	2.7 ± 0.0 ^{ab}	2.7 ± 0.0 ^{ab}	2.7 ± 0.0 ^{ab}	2.7 ± 0.0 ^{ab}	2.7 ± 0.0 ^{ab}	2.7 ± 0.0 ^{ab}	2.7 ± 0.0 ^{ab}	2.7 ± 0.0 ^{ab}
18:1n-9	9.1 ± 0.2 ^d	10.6 ± 0.1 ^{cd}	11.0 ± 0.5 ^{cd}	13.7 ± 0.2 ^b	11.7 ± 0.3 ^c	11.7 ± 0.3 ^c	13.7 ± 0.2 ^b	12.0 ± 0.2 ^c	11.3 ± 0.3 ^{cd}	20.4 ± 0.5 ^a	20.4 ± 0.5 ^a	20.4 ± 0.5 ^a	20.4 ± 0.5 ^a	20.4 ± 0.5 ^a	20.4 ± 0.5 ^a	20.4 ± 0.5 ^a	20.4 ± 0.5 ^a	20.4 ± 0.5 ^a
MUFA ²	24.5 ± 0.2 ^{cd}	22.4 ± 0.2 ^{cde}	24.9 ± 0.2 ^c	23.4 ± 0.2 ^{de}	28.0 ± 0.9 ^{de}	28.0 ± 0.9 ^{de}	23.4 ± 0.2 ^{de}	21.4 ± 0.3 ^e	23.3 ± 0.7 ^{de}	33.9 ± 0.6 ^a	33.9 ± 0.6 ^a	33.9 ± 0.6 ^a	33.9 ± 0.6 ^a	33.9 ± 0.6 ^a	33.9 ± 0.6 ^a	33.9 ± 0.6 ^a	33.9 ± 0.6 ^a	33.9 ± 0.6 ^a
18:2n-6	9.1 ± 0.2 ^e	9.3 ± 0.0 ^e	9.3 ± 0.1 ^c	30.2 ± 0.3 ^a	19.5 ± 0.7 ^b	19.5 ± 0.7 ^b	30.2 ± 0.3 ^a	13.9 ± 0.1 ^c	11.9 ± 0.3 ^d	15.1 ± 0.1 ^c	15.1 ± 0.1 ^c	15.1 ± 0.1 ^c	15.1 ± 0.1 ^c	15.1 ± 0.1 ^c	15.1 ± 0.1 ^c	15.1 ± 0.1 ^c	15.1 ± 0.1 ^c	15.1 ± 0.1 ^c
20:4n-6	0.9 ± 0.0 ^a	0.7 ± 0.0 ^{cd}	0.9 ± 0.0 ^a	0.6 ± 0.0 ^d	0.8 ± 0.0 ^{bc}	0.8 ± 0.0 ^{bc}	0.6 ± 0.0 ^d	0.6 ± 0.0 ^d	0.7 ± 0.0 ^{cd}	0.7 ± 0.0 ^{bc}	0.7 ± 0.0 ^{bc}	0.7 ± 0.0 ^{bc}	0.7 ± 0.0 ^{bc}	0.7 ± 0.0 ^{bc}	0.7 ± 0.0 ^{bc}	0.7 ± 0.0 ^{bc}	0.7 ± 0.0 ^{bc}	0.7 ± 0.0 ^{bc}
n-6 ³	10.7 ± 0.2 ^e	10.8 ± 0.0 ^e	11.0 ± 0.2 ^e	32.0 ± 0.3 ^a	21.2 ± 0.6 ^b	21.2 ± 0.6 ^b	32.0 ± 0.3 ^a	15.4 ± 0.2 ^c	13.4 ± 0.3 ^d	16.7 ± 0.1 ^c	16.7 ± 0.1 ^c	16.7 ± 0.1 ^c	16.7 ± 0.1 ^c	16.7 ± 0.1 ^c	16.7 ± 0.1 ^c	16.7 ± 0.1 ^c	16.7 ± 0.1 ^c	16.7 ± 0.1 ^c
18:3n-3	2.2 ± 0.0 ^e	2.3 ± 0.1 ^c	2.3 ± 0.0 ^c	2.1 ± 0.0 ^c	2.0 ± 0.0 ^c	2.0 ± 0.0 ^c	2.1 ± 0.0 ^c	23.7 ± 0.2 ^a	13.8 ± 1.2 ^b	2.4 ± 0.1 ^c	2.4 ± 0.1 ^c	2.4 ± 0.1 ^c	2.4 ± 0.1 ^c	2.4 ± 0.1 ^c	2.4 ± 0.1 ^c	2.4 ± 0.1 ^c	2.4 ± 0.1 ^c	2.4 ± 0.1 ^c
18:4n-3	3.1 ± 0.1 ^a	2.4 ± 0.1 ^b	2.6 ± 0.1 ^b	1.8 ± 0.0 ^d	2.4 ± 0.1 ^b	2.4 ± 0.1 ^b	1.8 ± 0.0 ^d	1.7 ± 0.1 ^d	2.2 ± 0.1 ^{bc}	1.9 ± 0.1 ^{cd}	1.9 ± 0.1 ^{cd}	1.9 ± 0.1 ^{cd}	1.9 ± 0.1 ^{cd}	1.9 ± 0.1 ^{cd}	1.9 ± 0.1 ^{cd}	1.9 ± 0.1 ^{cd}	1.9 ± 0.1 ^{cd}	1.9 ± 0.1 ^{cd}
20:5n-3	13.2 ± 0.1 ^a	10.0 ± 0.2 ^d	11.9 ± 0.2 ^{ab}	8.1 ± 0.1 ^e	11.3 ± 0.3 ^{bcd}	11.3 ± 0.3 ^{bcd}	8.1 ± 0.1 ^e	7.4 ± 0.2 ^e	10.2 ± 0.3 ^{cd}	7.8 ± 0.3 ^{bc}	7.8 ± 0.3 ^{bc}	7.8 ± 0.3 ^{bc}	7.8 ± 0.3 ^{bc}	7.8 ± 0.3 ^{bc}	7.8 ± 0.3 ^{bc}	7.8 ± 0.3 ^{bc}	7.8 ± 0.3 ^{bc}	7.8 ± 0.3 ^{bc}
22:5n-3	3.1 ± 0.1 ^a	2.5 ± 0.2 ^{abc}	3.0 ± 0.1 ^{ab}	2.1 ± 0.1 ^{cd}	2.6 ± 0.1 ^{abc}	2.6 ± 0.1 ^{abc}	2.1 ± 0.1 ^{cd}	1.8 ± 0.1 ^d	2.5 ± 0.1 ^{bc}	1.9 ± 0.1 ^d	1.9 ± 0.1 ^d	1.9 ± 0.1 ^d	1.9 ± 0.1 ^d	1.9 ± 0.1 ^d	1.9 ± 0.1 ^d	1.9 ± 0.1 ^d	1.9 ± 0.1 ^d	1.9 ± 0.1 ^d
22:6n-3	15.3 ± 0.3 ^a	11.3 ± 0.2 ^{abcd}	12.7 ± 0.3 ^b	9.6 ± 0.3 ^{cde}	11.8 ± 0.4 ^{bc}	11.8 ± 0.4 ^{bc}	9.6 ± 0.3 ^{cde}	8.5 ± 0.3 ^e	11.2 ± 0.4 ^{bcd}	9.1 ± 0.5 ^{de}	9.1 ± 0.5 ^{de}	9.1 ± 0.5 ^{de}	9.1 ± 0.5 ^{de}	9.1 ± 0.5 ^{de}	9.1 ± 0.5 ^{de}	9.1 ± 0.5 ^{de}	9.1 ± 0.5 ^{de}	9.1 ± 0.5 ^{de}
n-3 ⁴	38.6 ± 0.6 ^{bc}	29.9 ± 0.5 ^{de}	34.2 ± 0.6 ^{cd}	24.8 ± 0.5 ^{ef}	31.5 ± 0.9 ^d	31.5 ± 0.9 ^d	24.8 ± 0.5 ^{ef}	44.5 ± 0.7 ^a	41.5 ± 1.4 ^{ab}	24.2 ± 1.0 ^f	24.2 ± 1.0 ^f	24.2 ± 1.0 ^f	24.2 ± 1.0 ^f	24.2 ± 1.0 ^f	24.2 ± 1.0 ^f	24.2 ± 1.0 ^f	24.2 ± 1.0 ^f	24.2 ± 1.0 ^f
PUFA ⁵	49.7 ± 0.5 ^{cd}	41.1 ± 0.5 ^{ef}	45.7 ± 0.5 ^{def}	57.1 ± 0.4 ^{ab}	53.1 ± 0.5 ^{bc}	53.1 ± 0.5 ^{bc}	57.1 ± 0.4 ^{ab}	60.1 ± 0.5 ^a	55.3 ± 1.6 ^{ab}	41.2 ± 1.0 ^f	41.2 ± 1.0 ^f	41.2 ± 1.0 ^f	41.2 ± 1.0 ^f	41.2 ± 1.0 ^f	41.2 ± 1.0 ^f	41.2 ± 1.0 ^f	41.2 ± 1.0 ^f	41.2 ± 1.0 ^f
LC-PUFA ⁶	34.4 ± 0.6 ^a	26.2 ± 0.4 ^b	30.4 ± 0.5 ^b	21.7 ± 0.5 ^c	28.0 ± 0.9 ^b	28.0 ± 0.9 ^b	21.7 ± 0.5 ^c	19.8 ± 0.5 ^c	26.3 ± 0.5 ^b	20.9 ± 0.9 ^c	20.9 ± 0.9 ^c	20.9 ± 0.9 ^c	20.9 ± 0.9 ^c	20.9 ± 0.9 ^c	20.9 ± 0.9 ^c	20.9 ± 0.9 ^c	20.9 ± 0.9 ^c	20.9 ± 0.9 ^c
MC-PUFA ⁷	14.8 ± 0.2 ^f	14.4 ± 0.1 ^f	14.7 ± 0.5 ^f	34.4 ± 0.4 ^b	24.2 ± 0.6 ^d	24.2 ± 0.6 ^d	34.4 ± 0.4 ^b	39.6 ± 0.2 ^a	28.4 ± 1.4 ^c	19.7 ± 0.2 ^e	19.7 ± 0.2 ^e	19.7 ± 0.2 ^e	19.7 ± 0.2 ^e	19.7 ± 0.2 ^e	19.7 ± 0.2 ^e	19.7 ± 0.2 ^e	19.7 ± 0.2 ^e	19.7 ± 0.2 ^e
n-3:n-6	3.6 ± 0.1 ^a	2.8 ± 0.0 ^{bc}	3.1 ± 0.1 ^b	0.8 ± 0.0 ^e	1.5 ± 0.1 ^d	1.5 ± 0.1 ^d	0.8 ± 0.0 ^e	2.9 ± 0.1 ^{bc}	3.1 ± 0.1 ^b	1.4 ± 0.1 ^d	1.4 ± 0.1 ^d	1.4 ± 0.1 ^d	1.4 ± 0.1 ^d	1.4 ± 0.1 ^d	1.4 ± 0.1 ^d	1.4 ± 0.1 ^d	1.4 ± 0.1 ^d	1.4 ± 0.1 ^d
Total triacylglycerols (% of total lipid)	10.3 ± 1.1	10.8 ± 1.6	11.5 ± 1.6	9.7 ± 1.6	8.4 ± 1.6	8.4 ± 1.6	9.7 ± 1.6	10.1 ± 1.6	8.0 ± 1.6	8.3 ± 1.6	8.3 ± 1.6	8.3 ± 1.6	8.3 ± 1.6	8.3 ± 1.6	8.3 ± 1.6	8.3 ± 1.6	8.3 ± 1.6	8.3 ± 1.6

Values represent least-square means (relative area % of FAME) ± SE of two individual samples from all tanks. Means within fatty acid(s) with common letter superscripts are not significantly different. Absence of superscripts indicates lack of statistical significance

¹⁻⁷ All superscripts denote information provided as footnotes in Table 3

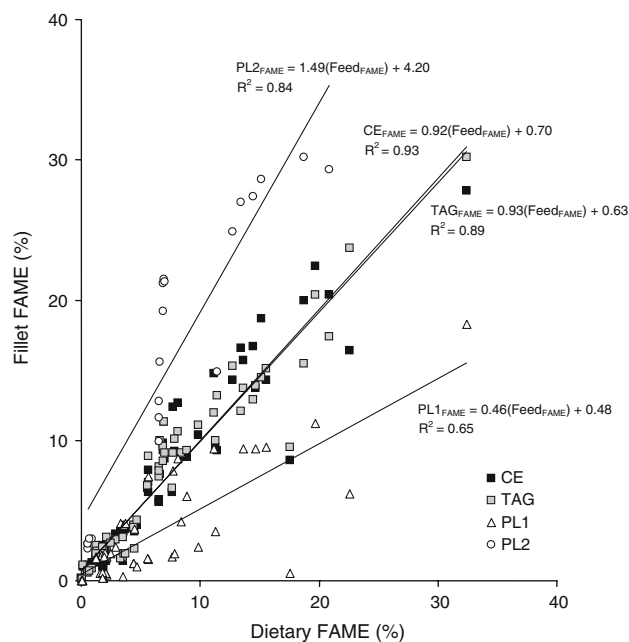


Fig. 1 Relationship between mean fatty acid composition (as relative % of fatty acid methyl esters) of feeds and fillet lipid classes. All fatty acids listed in Tables 1, 2, 3 are depicted. Abbreviations: *CE* cholesteryl esters, *TAG* triacylglycerols, *PL1* phospholipids, *16:0* 20:4n-6, 20:5n-3, 22:6n-3, *PL2* phospholipids, all other fatty acids. *Solid lines* represent best-fit linear regressions; equations and R^2 values are provided for each regression

(specifically 18:2n-6, 18:3n-3, and 18:4n-3) and n-6 FA (Table 6). CE was the most predominant lipid class represented within the fillet total lipid (~70% of total lipid, Table 4), and was highest in MUFA (specifically 18:1n-7 and 18:1n-9, Table 6).

Discussion

Fatty acid composition of fillet lipid classes was affected by feeding regimen, however, the extent to which tissue profile reflected dietary profile varied among lipid classes and feeding regimens. Fillet TAG and CE profiles of unfinished groups mirrored dietary FA composition in a direct relationship approaching unity (Fig. 1), indicating FA profile within these classes is plastic and suggests their composition is established via nonspecific processes. Accordingly, the neutral lipid classes were quite responsive to finishing, and overall, demonstrated a high level of finishing success, that is, restoration of the FO-associated FA profile. However, in terms of total LC-PUFA content within the CE, only the CO + Finish and the PO + Finish regimens were equivalent to the FO Control regimen. Of the experimental regimens, CO + Finish and PO + Finish were also the highest in LC-PUFA within the TAG

Table 6 Fatty acid (FA) composition of fillet lipid classes, with respect to predominant (>1% fatty acid methyl esters, FAME) fatty acid (FA) and FA classes, pooled across feeding regimens

Fatty acid(s)	Lipid class		
	Cholesteryl esters	Phospholipids	Triacylglycerols
12:0	1.5 ± 0.0 ^b	0.1 ± 0.0 ^c	1.6 ± 0.0 ^a
14:0	5.5 ± 0.0 ^b	1.7 ± 0.0 ^c	5.8 ± 0.0 ^a
16:0	18.7 ± 0.1 ^b	29.0 ± 0.1 ^a	14.7 ± 0.1 ^c
18:0	3.5 ± 0.0 ^b	4.0 ± 0.0 ^a	1.9 ± 0.0 ^c
SFA ¹	30.1 ± 0.2 ^b	35.5 ± 0.2 ^a	24.8 ± 0.2 ^c
16:1n-7	8.4 ± 0.1 ^b	2.1 ± 0.1 ^c	9.3 ± 0.1 ^a
18:1n-7	2.8 ± 0.0 ^a	2.1 ± 0.0 ^c	2.5 ± 0.0 ^b
18:1n-9	14.8 ± 0.1 ^a	8.8 ± 0.1 ^c	12.8 ± 0.1 ^b
MUFA ²	26.9 ± 0.2 ^a	13.3 ± 0.2 ^c	25.2 ± 0.2 ^b
18:2n-6	13.6 ± 0.1 ^b	7.9 ± 0.1 ^c	14.5 ± 0.1 ^a
20:4n-6	1.1 ± 0.0 ^b	2.8 ± 0.0 ^a	0.8 ± 0.0 ^c
n-6 ³	15.7 ± 0.1 ^b	11.5 ± 0.1 ^c	16.1 ± 0.1 ^a
18:3n-3	4.0 ± 0.1 ^b	1.2 ± 0.1 ^c	5.9 ± 0.1 ^a
18:4n-3	1.1 ± 0.0 ^b	0.2 ± 0.0 ^c	2.3 ± 0.0 ^a
20:5n-3	7.6 ± 0.1 ^c	13.7 ± 0.1 ^a	10.2 ± 0.1 ^b
22:5n-3	1.8 ± 0.0 ^b	1.6 ± 0.0 ^c	2.5 ± 0.0 ^a
22:6n-3	11.5 ± 0.2 ^b	22.3 ± 0.2 ^a	11.3 ± 0.2 ^b
n-3 ⁴	27.0 ± 0.3 ^c	39.5 ± 0.3 ^a	33.5 ± 0.3 ^b
PUFA ⁵	43.0 ± 0.3 ^c	51.2 ± 0.3 ^a	50.0 ± 0.3 ^b
LC-PUFA ⁶	23.1 ± 0.3 ^c	41.0 ± 0.3 ^a	26.3 ± 0.3 ^b
MC-PUFA ⁷	19.0 ± 0.2 ^b	9.6 ± 0.2 ^c	23.0 ± 0.2 ^a
n-3:n-6	2.0 ± 0.0 ^c	3.8 ± 0.0 ^a	2.4 ± 0.0 ^b

Values represent least-square means

Standard errors less than ±0.1 are represented as ±0.0

¹⁻⁷ All superscripts denote information provided as footnotes in Table 3

fraction; however, within this lipid class they remained statistically different from the FO Control regimen.

Fillet PL composition was less responsive to dietary manipulations than the neutral lipid fractions, that is, the difference between finished and unfinished profiles was generally less noticeable within the PL fraction compared to the CE and TAG fractions. Although dietary FA composition was largely reflected within the PL, the relationship was more or less direct depending on the individual FA. For 16:0, 20:4n-6, 20:5n-3, and 22:6n-3, the relationship between dietary composition and tissue composition was greater than 1:1, suggesting selective incorporation of these FA within the PL fraction (Fig. 1). Conversely, for all other FA, the relationship was less than 1:1, suggesting exclusion of these FA from the PL fraction.

In comparing the finished and unfinished groups, it is interesting to note the pattern of change which did occur within the PL fraction. In the case of the GO-fed fish, the GO group displays a high level of 18:2n-6 enrichment

within the PL, but the level of 18:2n-6 in the finished group is less than half that observed in the unfinished fish. In comparing the LO- and PO-fed regimens, we observe a similar PL enrichment in 18:3n-3 and 18:1n-9 among the LO and PO groups, respectively, and comparable reductions in these FA within the PL of the finished groups. Within the CO-fed fish, there is virtually no enrichment of 12:0 within the PL, and only a minor distinction between the final PL profiles of the CO and CO + Finish regimens. This suggests some level of plasticity within the PL fraction, but perhaps more importantly, a selectivity for certain FA which may drive profile change within this lipid class.

Phospholipids have been described as being less responsive to dietary manipulations than neutral lipids [6], but also as exhibiting more active FA turnover than neutral lipid [9, 20, 21]. The specificity of PL for FA of certain chain lengths and degrees of unsaturation has been documented [22], and may be responsible for the discrepancy between differing observations of unresponsiveness and active profile change within the PL fraction. Specificity of PL for certain FA moieties is thought to arise from FA selectivity occurring during two processes related to PL biosynthesis, specifically *de novo* phosphatidic acid synthesis and phospholipid FA remodeling. In terms of *de novo* phosphatidic acid synthesis, for example, mitochondrial glycerol-3-phosphate acyltransferase preferentially utilizes saturated FA acyl-coAs (acylated to the sn-1 position), whereas lysophosphatidic acid acyltransferase has a greater affinity for unsaturated FA acyl-coAs (acylated to the sn-2 position). Further, the FA binding proteins (FABP), which apparently direct this process via inhibitory/stimulatory effects on key enzymes and by regulating FA absorption and cellular uptake, also demonstrate FA selectivity. Fatty acid binding proteins, and their associated FA specificities, are also involved in the process of PL remodeling via deacylation-reacylation and transacylation reactions [22].

The FA composition of PL fractions in fishes may also be determined, in part, by the affinity of acyl transferases and/or FABPs for particular FA moieties. In fishes, the PL fraction is commonly associated with 16:0 and LC-PUFA, in a variety of tissues, including gametes [23, 24], and neural, optic, and immunocompetent cells [25], and our data are in agreement with these observations. In Atlantic salmon *Salmo salar* hepatocytes, 16:0, 20:4n-6, 20:5n-3, 22:6n-3 were preferentially incorporated into intra- and extracellular polar fractions relative to 18:1n-9, 18:2n-6, and 18:3n-3 [26]. Thus, the processes of FA incorporation and/or remodeling within the PL fraction of fish appear selective and similar to those observed in mammals. However, the prevalence of certain molecular “species” of PL is also influenced by FA availability, that is, dietary intake. Hvattum et al. [25] quantified the molecular species

of PL within head kidney macrophages of Atlantic salmon reared on diets containing FO, soybean oil, or a 50:50 blend of these two lipids. Among fish fed the soybean oil diet, increasing dietary abundance of 18:2n FA (the analytical methods used in this study do not allow position of double bonds to be assigned, but the presumption is that 18:2n represents 18:2n-6, 20:5n represents 20:5n-3, 22:6n represents 22:6n-3, etc.) was reflected within the PL. Although some C₂₀ and C₂₂-containing species decreased with increasing dietary soybean oil (e.g., 14:0/22:6 and 16:0/20:5n PC, 16:0/22:6n and 16:0/20:5n-3 PE, 16:0/20:5n and 16:0/22:6n PI, 16:0/20:5n and 16:0/22:6n PS, etc.) others increased (18:2n/20:5n and 18:2n/22:6n PE, 18:2/22:6n PS, etc.). This suggests that, despite decreasing dietary availability, 20:5n-3 and 22:6n-3 were being actively retained in the polar lipid, but increasingly paired with abundant 18:2n-6. Bell et al. [25] also noted conservation of LC-PUFA within the PL of salmon head kidney macrophages, but similar to Hvattum et al. [27], these authors observed substantial enrichment of 18:3n-3 and 18:2n-6 when this FA was abundant in the feed. Interestingly, the magnitude of 18:3n-3 enrichment was lower than that of 18:2n-6 despite roughly equivalent dietary levels [25]. We also observed 18:2n-6 enrichment within the PL (18.3% in GO-fed fish vs. 32.40% in GO feed) to exceed that of 18:3n-3 relative to dietary content (6.2% in LO-fed fish vs. 22.56% in LO feed), suggesting selective process(es) in PL metabolism have a greater affinity for 18:2n-6 than 18:3n-3.

Generally, composition of the neutral lipid classes demonstrates less specificity for certain FA moieties. Although many of the same enzymes and proteins involved in PL metabolism are also associated with TAG synthesis and remodeling, apparently TAG fractions are not as strongly affected by FA selectivity [24]. FA specificity may occur within biosynthesis of CE; however, lecithin:cholesterol acyltransferase, the enzyme responsible for esterifying FA to cholesterol, shows a preference for C₁₄ and C₁₆ FA [28]. The data available for fishes supports extension of these mammalian paradigms to piscine models. Although Stubhaug et al. [26] noted significant differences in incorporation of certain FA within the intracellular neutral lipid of Atlantic salmon hepatocytes, in general, this fraction showed less specificity for individual FA than the polar fraction [26]. Specificity was noted within the extracellular neutral lipid, but in this case, selection was for 16:0 and 18:0 within the sterol esters and TAG, respectively. In general, we did not observe enrichment of FA within the neutral lipid beyond dietary composition, and thus our data do not support a hypothesis of positive selection for certain FA within the CE or TAG fractions. However, the shorter-chain SFA (8:0, 10:0, 12:0, and 14:0) content of the CE and TAG fractions of fish fed

the CO feed was lower than expected in all lipid classes, suggesting selection against deposition of these FA in fillet tissue.

High dietary levels of shorter-chain SFA in the CO feed were not proportionally reflected in fillet PL, TAG, or CE, or total lipid of other major lipid depots [5], which suggests either (1) impaired digestion/absorption or (2) catabolism of these FA. Lower digestibility of alternative lipids has been reported for cold-water marine species [29–32], and has been attributed to differences in melting points and PUFA content between the alternative lipid and FO. Reductions in growth are also observed in these cases, and dietary FA composition of poorly digested lipid is not reflected within the tissues [32]. Reduced digestibility of the CO feed seems an unlikely explanation for our results given the lack of growth differences and the limited, but significant, enrichment of CO-associated FA within the fillet and other tissues [5]. Sunshine bass can effectively utilize a wide-range of alternative lipids [3–5], as can other cool- and warm-water species, indicating lipid digestibility is primarily temperature-dependent, and issues of poor lipid digestion may be limited to cold-water species. Catabolism of shorter-chain SFA for energy production explains the low level of shorter-chain SFA enrichment observed in the CO-fed fish. Rates of β -oxidation have been determined for a variety of FA in several fish species, and these studies indicate SFA are preferred substrates for energy production [33–36].

The available data indicate selective processes govern the metabolic fate of FA in fishes. Although neutral lipids reflect dietary composition in a near-direct fashion, polar lipids show evidence of preference for certain FA. Medium-chain and LC-PUFA are preferred substrates for inclusion in polar lipid, whereas shorter-chain SFA are excluded. Although MC-PUFA appear to be somewhat inferior to LC-PUFA in terms of affinity, increased availability may overwhelm any enzymatic selectivity and result in high levels of incorporation of MC-PUFA within the PL fraction. Shorter-chain SFA are apparently poor substrates for the enzymes involved in PL synthesis and remodeling, and are not included within the PL fraction, even when availability is high. Conversely, SFA are preferentially used for energy production, whereas MC- and LC-PUFA are catabolized only when preferred substrates are absent. FA metabolism in fishes is characterized by a functional division between SFA and LC-PUFA, with MC-PUFA bridging the gap. Of the feeds we evaluated, the CO feed represented an ideal balance between providing preferred FA substrates for energy production (SFA) and tissue incorporation (LC-PUFA), and few MC-PUFA to compete within either process. We demonstrated provision of SFA in grow-out feeds for SB, instead of competing MC-PUFA, meets energy demands and allows

for maximum inclusion of LC-PUFA within fillet lipid classes.

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