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Saturated Lipid Sources in Feeds for Sunshine Bass: Alterations in Production Performance and Tissue Fatty Acid Composition

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Abstract.—Feeds rich in saturated (SFAs) and monounsaturated fatty acids (MUFA) appear to maximize the retention of long-chain polyunsaturated fatty acids (LC-PUFA) in the fillets of sunshine bass (female white bass *Morone chrysops* × male striped bass *M. saxatilis*). To determine whether different sources of SFA and MUFA have equivalent effects on tissue fatty acid (FA) profile change, coconut (CO) and palm oils(PO) were evaluated as partial and complete substitutes for fish oil (FO) in feeds for juvenile sunshine bass. After 8 weeks of culture, the production performance of all groups was within the acceptable range for sunshine bass; however, weight gain was significantly reduced within the 100%-CO group. Partial replacement of FO with CO or PO resulted in significant alteration of fillet FA profiles, and these changes were exacerbated in the complete-FO-replacement groups. The LC-PUFA were disproportionately enriched in the fillets of fish fed the reduced or FO-free feeds, whereas dietary surpluses of 12:0 and 14:0 (CO-based feeds) and 16:0 (PO-based feeds) were not reflected in any of the tissues analyzed. Rather, the levels of the SFA elongation–desaturation products (i.e., 18:0 and 18:1[n-9]) were elevated, particularly within liver lipid, suggesting enhanced FA biotransformation activity among fish fed higher levels of either alternative lipid source. Based on tissue FA composition, PO and CO appear to be approximately equivalent in terms of fillet LC-PUFA retention. However, the digestibility and utilization of these feedstuffs must be further investigated to maximize the ability of PO and CO to (partially or completely) replace FO in sunshine bass feeds.

To sustain long-term growth in the aquafeed and aquaculture industries, development and application of reduced or fish oil (FO)-free feed formulations is needed. Although use of plant- and terrestrial animal-derived lipids is attractive in terms of reducing the economic and environmental costs of aquafeeds, replacing FO with alternative lipid sources reduces long-chain polyunsaturated fatty acid (LC-PUFA) content of the feeds. Because fish tissues respond in a time-dependent manner to changes in fatty acid (FA) intake (Jobling 2003, 2004a, 2004b; Robin et al. 2003), FO replacement reduces deposition of these functional nutrients within the resultant fillets.

Retention of LC-PUFA within the fillet lipid can be influenced by the FA composition of the feed used during grow-out (Trushenski et al. 2008a, 2009). Specifically, LC-PUFA retention is greater among fish raised on feeds high in saturated (SFA; degree of unsaturation = 0) and monounsaturated FAs (MUFA; degree of unsaturation = 1) compared with those fed medium-chain polyunsaturated FAs (MC-PUFA; chain length = 18 carbon atoms, degree of unsaturation ≥2). However, it is unclear whether different sources of dietary SFA and MUFA are equivalent in this respect.

To address whether specific composition of SFA- and MUFA-rich lipids influences tissue fatty acid (FA) profile change, I evaluated partial and complete replacement of FO with coconut oil (CO) and palm oil (PO) in feeds for juvenile sunshine bass (female white bass *Morone chrysops* × male striped bass *M. saxatilis*).

Methods

Feeds and feeding trial.—A practical sunshine bass feed (Table 1), including 200 g/kg (dry matter basis) menhaden meal (Special Select, Omega Protein, Inc., Houston, Texas) and 98 g/kg menhaden fish oil (100% FO, Virginia Gold, Omega Protein, Inc., Houston, Texas), served as the control dietary formulation in the present work. Four experimental feeds were derived from the basal formulation, with 50% or 100% of the FO replaced with PO (50% PO, 100% PO; Jungle Products, Edison, New Jersey) or CO (50% CO, 100% CO; Spectrum Organic Products, Petaluma, California). All feedstuffs were incorporated using a cutter–mixer (model CM450, Hobart Corporation, Troy, Ohio), pelleted using a food grinder, dried at room temperature to approximately 889 g/kg dry matter, and stored frozen (−20°C) throughout the duration of the study. Proximate analyses of triplicate diet samples were conducted according to standard methods for analysis of animal feeds (Folch et al. 1957; AOAC 1995) to confirm diet

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TABLE 1.—Formulation and proximate composition of experimental feeds fed to sunshine bass. All proximate composition values are expressed as means ± SEs (g/kg; dry matter basis).

Ingredient	100% Fish oil	50% Coconut oil	100% Coconut oil	50% Palm oil	100% Palm oil
Fish meal[a]	200	200	200	200	200
Fish oil[a]	98	49		49	
Coconut oil[b]		49	98		
Palm oil[c]				49	98
Corn gluten meal[d]	140	140	140	140	140
Wheat bran	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[e]	1.5	1.5	1.5	1.5	1.5
Vitamin premix[f]	1.5	1.5	1.5	1.5	1.5
Proximate composition					
Dry matter	883 ± 1	890 ± 1	893 ± 1	893 ± 1	884 ± 1
Protein	405 ± 8	409 ± 8	394 ± 8	401 ± 8	405 ± 8
Lipid	146 ± 2	151 ± 2	137 ± 2	147 ± 2	151 ± 2
Ash	104 ± 1	104 ± 1	104 ± 1	103 ± 1	102 ± 1

[a] Derived from menhaden *Brevoortia* spp.; Omega Protein, Inc., Houston, Texas.
[b] Spectrum Organic Products, Petaluma, California.
[c] Jungle Products, Edison, New Jersey.
[d] Tate and Lyle, Decatur, Illinois.
[e] Formulated to contain (per kilogram) 7,000 mg copper, 70,000 mg iron, 100,000 mg manganese, and 200,000 mg zinc, along with 0.24% iodine.
[f] Formulated to contain (per kilogram) 99.8 mg selenium, 2,200 mg folic acid, 88,000 mg niacin, 35,200 mg pantothenic acid, 11,000 mg vitamin B_6, 13,200 mg riboflavin, 11,000 mg thiamin, 11,000 mg vitamin B_{12}, 66,000 mg vitamin E, 4,400 mg vitamin K, 4,400,000 IU vitamin A, 2,200,000 IU vitamin D, and 100,000 mg vitamin C.

composition (Table 1). Reserved crude lipid samples were analyzed for FA composition (Table 2) according to the procedures described by Lane et al. (2006).

Experimental design and feeding trial.—Twenty-five juvenile sunshine bass (25.4 ± 0.1 g [mean ± SE]; Keo Fish Farm, Keo, Arkansas) were stocked into each tank of a recirculation system consisting of thirty 270-L fiberglass tanks with associated mechanical and biological filtration units. Each experimental feed was randomly assigned to six replicate tanks; however, a mechanical failure resulted in the loss of one tank within the 100%-CO treatment group ($N = 6$ for 100% FO, 50% CO, 50% PO, and 100% PO; $N = 5$ for 100% CO). All fish were fed assigned feeds once daily to apparent satiation to simplify trial management.

Temperature, dissolved oxygen (YSI Model 55 Oxygen Meter, Yellow Springs, Ohio), ammonia-, nitrite-, and nitrate-nitrogen as well as alkalinity were measured periodically throughout the study period (Hach DR/2010 spectrophotometer, Hach Company, Loveland, Colorado). All water quality conditions were maintained within ranges suitable for sunshine bass culture (Kohler 2000). All culture and husbandry methods, as well as euthanasia and sample collection procedures described below, were conducted under the direction and approval of the Southern Illinois University Institutional Animal Care and Use Committee, protocol 07–046.

Harvest, sample collection, and production performance.—After 8 weeks, two fish were removed from each tank and immediately anesthetized in a solution of tricaine methanesulfonate (MS-222; ~200 mg/L) in culture water. Each anesthetized fish was individually weighed and euthanized by single cranial pithing before tissue sample collection. Samples of fillet tissue were taken from the fillets, packaged in sterile polyethylene bags (Whirl-pak, Nasco, Fort Atkinson, Wisconsin), and stored frozen (−80°C) before proximate and FA analyses. Livers were dissected from the viscera to calculate hepatosomatic index [HSI; (liver weight/whole body weight) × 100]. Samples of liver and intraperitoneal fat (IPF) tissue were packaged and stored in the same manner as fillet samples for FA analysis.

Average individual percent weight gain {[(average individual weight$_{final}$ − average individual weight$_{initial}$)/average individual weight$_{initial}$] × 100}, feed conversion ratio [FCR, average individual dry matter (DM) consumption/average individual gain], feed intake {FI, [total DM intake/(initial weight × final weight)$^{0.5}$/d] × 100}, and specific growth rate {SGR, [log$_e$(final weight) − log$_e$(initial weight)]/d} were calculated for each tank. Excluding the mechanical failure resulting in the loss of one tank, no mortalities occurred during the 8-week culture period.

Fillet lipid composition.—Fillet samples were lyophilized and pulverized before lipid extraction and FA

TABLE 2.—Fatty acid composition of experimental feeds. Abbreviations are as follows: SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, MC-PUFA = medium-chain PUFA, and LC-PUFA = long-chain PUFA (see footnotes for additional details). In the notation for individual fatty acids, the number to the left of the colon is the number of carbon atoms in the compound, the number immediately to the right of the colon is the number of double bonds, and the number after the hyphen indicates the position of the first double bond from the methyl end.

Fatty acid(s)	100% Fish oil	50% Coconut oil	100% Coconut oil	50% Palm oil	100% Palm oil	SE
8:0	0.0	0.6	1.3	0.0	0.0	0.1
10:0	0.0	1.8	3.4	0.0	0.0	0.0
12:0	0.2	18.4	35.3	0.1	0.2	0.0
14:0	8.1	12.2	15.9	4.8	2.0	0.0
16:0	19.7	16.2	13.5	29.4	35.6	0.0
18:0	3.5	3.3	3.3	4.1	4.2	0.0
Total SFA[a]	33.1	53.8	73.3	39.7	43.0	0.1
16:1(n-7)	10.6	6.1	1.7	5.7	1.5	0.0
18:1(n-7)	2.9	1.8	0.7	1.9	1.0	0.0
18:1(n-9)	9.2	8.9	9.1	21.1	34.3	0.0
Total MUFA[b]	24.0	17.7	11.7	29.6	37.3	0.0
16:2(n-4)	1.2	0.7	0.2	0.7	0.2	0.0
16:3(n-4)	1.6	0.9	0.2	0.9	0.2	0.0
18:2(n-6)	8.8	8.4	9.3	11.8	14.5	0.1
20:4(n-6)	1.4	0.8	0.3	0.8	0.2	0.0
All (n-6)[c]	10.9	9.7	9.7	13.0	14.8	0.1
18:3(n-3)	1.6	1.1	0.7	1.2	0.8	0.0
18:4(n-3)	2.1	1.2	0.3	1.1	0.3	0.0
20:4(n-3)	1.2	0.7	0.2	0.6	0.1	0.0
20:5(n-3)	12.7	7.2	1.8	6.7	1.5	0.0
22:5(n-3)	2.5	1.4	0.3	1.3	0.3	0.0
22:6(n-3)	8.6	5.1	1.5	4.8	1.3	0.0
All (n-3)	28.7	16.9	4.8	15.9	4.4	0.0
Total PUFA[d]	43.0	28.6	15.0	30.7	19.7	0.1
Total LC-PUFA[e]	26.6	15.5	4.1	14.5	3.6	0.0
Total MC-PUFA[f]	13.4	11.3	10.4	14.6	15.7	0.1
(n-3):(n-6)	2.6	1.7	0.5	1.2	0.3	0.0

[a] Sum of all fatty acids without double bonds; includes 15:0 and 17:0 in addition to the individually reported SFA.
[b] Sum of all fatty acids with one double bond; includes 14:1, 17:1, and 24:1(n-9) in addition to the individually reported MUFA.
[c] Includes 18:3(n-6), 20:2(n-6), and 20:3(n-6) in addition to the individually reported (n-6) fatty acids.
[d] Sum of all fatty acids with two or more double bonds; includes 18:3(n-4), 20:4(n-3), 20:2(n-6), and 20:3(n-6) in addition to the individually reported PUFA.
[e] Sum of all PUFA with 20 or more carbon atoms, three or more double bonds; includes 20:3(n-6) in addition to the individually reported LC-PUFA.
[f] Sum of all PUFA with 18 carbon atoms; includes 18:3(n-6) in addition to the individually reported MC-PUFA.

analysis using the same methods as for diet samples (Lane et al. 2006). Intact liver and IPF samples were homogenized in solvent before extraction in the same manner as the other samples.

Statistical analyses.—All data were analyzed by one-way analysis of variance (ANOVA) within the general linear model framework of the Statistical Analysis System, version 9.1 (SAS Institute 2005) to determine significance of differences among dietary treatment regimes. For parameters exhibiting significant differences, means were compared using post hoc Tukey's Honest Significant Difference (HSD) tests. In all cases, differences were considered significant at $P < 0.05$. Regression of FA data were calculated using the regression function in Excel 2007 (Microsoft 2007).

Results

Partial replacement of FO with CO or PO resulted in significant alteration of fillet FA profile, and the alterations associated with 50%-FO replacement were exacerbated within the 100%-replacement groups (Table 3). Feeding increasing levels of CO resulted in increased fillet deposition of 12:0, 14:0 and total SFA, at the expense of 20:5(n-3), 22:6(n-3), and LC-PUFA.[1]

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[1] In the fatty acid notation used in this paper, the number to the left of the colon is the number of carbon atoms in the compound, the number immediately to the right of the colon is the number of double bonds, and the number after the hyphen indicates the position of the first double bond from the methyl end.

TABLE 3.—Total fatty acid composition of sunshine bass fillets. Values represent least-squares means of duplicate samples from five or more replicate tanks. Means with letters in common are not significantly different. SE values <0.1 are reported as 0.0. See Table 2 for additional details.

Fatty acid(s)	100% Fish oil	50% Coconut oil	100% Coconut oil	50% Palm oil	100% Palm oil	P-value
12:0	0.2 ± 0.2 x	8.8 ± 0.2 y	14.6 ± 0.2 z	0.1 ± 0.2 x	0.2 ± 0.2 x	<0.001
14:0	5.6 ± 0.1 x	8.9 ± 0.1 y	11.4 ± 0.1 z	3.5 ± 0.1 w	1.8 ± 0.1 v	<0.001
16:0	20.0 ± 0.2 x	18.6 ± 0.2 w	18.2 ± 0.2 w	23.0 ± 0.2 y	25.1 ± 0.2 z	<0.001
18:0	3.7 ± 0.1 y	3.7 ± 0.1 y	4.3 ± 0.1 z	3.8 ± 0.1 y	4.4 ± 0.1 z	<0.001
Total SFA[a]	30.4 ± 0.4 w	41.0 ± 0.4 y	49.4 ± 0.4 z	31.1 ± 0.4 xw	31.9 ± 0.4 x	<0.001
16:1(n-7)	9.4 ± 0.1 z	6.4 ± 0.1 y	3.5 ± 0.1 x	6.4 ± 0.1 y	3.1 ± 0.1 w	<0.001
18:1(n-7)	3.3 ± 0.0 z	2.3 ± 0.0 y	1.3 ± 0.0 x	2.3 ± 0.0 y	1.4 ± 0.0 x	<0.001
18:1(n-9)	12.9 ± 0.3 v	14.7 ± 0.3 w	19.3 ± 0.4 x	25.2 ± 0.3 y	35.4 ± 0.3 z	<0.001
20:1(n-9)	1.0 ± 0.0 y	0.9 ± 0.0 y	1.0 ± 0.0 y	1.1 ± 0.2 z	1.2 ± 0.2 z	<0.001
Total MUFA[b]	27.3 ± 0.4 x	25.2 ± 0.4 w	26.0 ± 0.5 w	35.6 ± 0.4 y	41.5 ± 0.4 z	<0.001
18:2(n-6)	8.3 ± 0.2 w	9.7 ± 0.2 x	11.4 ± 0.2 y	11.9 ± 0.2 y	15.4 ± 0.2 z	<0.001
20:4(n-6)	1.9 ± 0.0 z	1.5 ± 0.0 y	1.2 ± 0.0 x	1.3 ± 0.0 x	0.9 ± 0.0 w	<0.001
All (n-6)[c]	11.4 ± 0.2 w	12.4 ± 0.2 x	14.3 ± 0.2 y	14.3 ± 0.2 y	17.5 ± 0.2 z	<0.001
18:3(n-3)	1.3 ± 0.0 z	1.1 ± 0.0 y	0.9 ± 0.0 x	1.1 ± 0.0 y	0.8 ± 0.0 x	<0.001
18:4(n-3)	1.3 ± 0.0 z	0.8 ± 0.0 y	0.3 ± 0.0 x	0.7 ± 0.0 w	0.2 ± 0.0 v	<0.001
20:4(n-3)	1.0 ± 0.0 z	0.7 ± 0.0 y	0.3 ± 0.0 x	0.6 ± 0.0 x	0.2 ± 0.0 v	<0.001
20:5(n-3)	11.0 ± 0.1 z	7.5 ± 0.1 y	3.5 ± 0.2 w	6.7 ± 0.2 x	2.9 ± 0.2 v	<0.001
22:5(n-3)	2.9 ± 0.0 z	2.1 ± 0.0 y	0.9 ± 0.0 w	1.7 ± 0.0 x	0.8 ± 0.0 v	<0.001
22:6(n-3)	11.1 ± 0.2 z	7.6 ± 0.2 y	4.0 ± 0.2 w	6.8 ± 0.2 x	3.8 ± 0.2 w	<0.001
All (n-3)	28.6 ± 0.4 z	19.9 ± 0.4 y	9.8 ± 0.4 w	17.7± 0.4 x	8.7 ± 0.4 w	<0.001
Total PUFA[d]	42.2 ± 0.5 z	33.8 ± 0.5 y	24.6 ± 0.5 w	33.2 ± 0.5 y	26.6 ± 0.5 x	<0.001
Total LC-PUFA[e]	28.2 ±0.4 z	19.7 ± 0.4 y	10.1 ± 0.5 w	17.3 ± 0.4 x	8.8 ± 0.4 v	<0.001
Total MC-PUFA[f]	11.7 ± 0.2 v	12.3 ± 0.2 w	13.2 ± 0.2 x	14.3 ± 0.2 y	16.9 ± 0.2 z	<0.001
(n-3):(n-6)	2.5 ± 0.0 z	1.6 ± 0.0 y	0.7 ± 0.0 w	1.2 ± 0.0 x	0.5 ± 0.0 v	<0.001
Total lipid[g]	9.1 ± 0.5 y	9.7 ± 0.5 y	9.1 ± 0.5 y	11.3 ± 0.5 z	8.9 ± 0.5 y	0.008

[a] Includes 10:0, 15:0, and 17:0 in addition to the individually reported SFA.

[b] Includes 14:1, 17:1, and 24:1(n-9) in addition to the individually reported MUFA.

[c] Includes 18:3(n-6), 20:2(n-6), and 20:3(n-6) in addition to the individually reported (n-6) fatty acids.

[d] Includes 16:2(n-4), 16:3(n-4), 18:3(n-4), 20:4(n-3), 20:2(n-6), and 20:3(n-6) in addition to the individually reported PUFA.

[e] Includes 20:3(n-6) in addition to the individually reported LC-PUFA.

[f] Includes 18:3(n-4) and 18:3(n-6) in addition to the individually reported MC-PUFA.

[g] Percent of dry matter.

Feeding PO was also associated with reductions in fillet LC-PUFA and significant enrichment of PO-associated FAs within the total lipid profile, most notably 18:1(n-9) and total MUFA. Liver and IPF FA profiles were also affected by dietary FA composition (Tables 4, 5). All tissues essentially mirrored dietary composition within the 100%-FO, 50%-CO, and 50%-PO groups; however, notable deviations from dietary profile were observed in the 100%-CO and 100%-PO groups, particularly within the fillet and liver lipid (Figures 1, 2).

Production performance was generally within acceptable ranges for sunshine bass; however, weight gain was significantly reduced within the 100%-CO group (Table 6). Food conversion ratio, FI, and SGR were not significantly affected by dietary treatment. The HSI was significantly elevated within the 100%-FO replacement groups; corresponding increases in liver lipid were also observed (Table 4).

Discussion

Divergence in tissue FA profile among fish fed different lipid sources has been widely reported (Bell et al. 2001, 2002; Caballero et al. 2002; Bransden et al. 2003; Glencross et al. 2003; Wonnacott et al. 2004; Lane et al. 2006; Menoyo et al. 2007; Lewis and Kohler 2008), and in this sense, our observations of varying fillet FA composition are conventional. However, there are several noteworthy nuances in the present dataset that provide further insight. First, the magnitude of the differences between treatment groups is considerable and developed over a relatively short period of time. We evaluated the 50%-CO formulation in a previous trial with sunshine bass (Trushenski et al. 2008a), and comparing the results of this previous study with the present work provides a useful illustration of this point. Previously, we observed significant fillet enrichment of SFAs after feeding for 20 weeks, but did not observe a significant reduction of LC-PUFA until 28 weeks had

TABLE 4.—Total fatty acid composition of the livers of sunshine bass. See Table 3 for additional details.

Fatty acid(s)	100% Fish oil	50% Coconut oil	100% Coconut oil	50% Palm oil	100% Palm oil	P-value
12:0	0.1 ± 0.2 x	2.4 ± 0.2 y	5.3 ± 0.2 z	0.0 ± 0.2 x	0.0 ± 0.2 x	<0.001
14:0	2.8 ± 0.2 x	5.7 ± 0.2 y	10.4 ± 0.2 z	2.3 ± 0.2 xw	2.1 ± 0.2 w	<0.001
16:0	14.7 ± 0.3 w	16.3 ± 0.3 x	19.4 ± 0.4 z	18.1 ± 0.3 y	18.9 ± 0.3 zy	<0.001
18:0	5.5 ± 0.6 x	6.6 ± 0.6 yx	9.5 ± 0.6 z	7.5 ± 0.6 y	9.8 ± 0.6 z	<0.001
Total SFA[a]	23.2 ± 0.8 w	31.3 ± 0.8 y	45.0 ± 0.9 z	28.0 ± 0.8 x	31.0 ± 0.8 y	<0.001
16:1(n-7)	5.7 ± 0.2 z	4.5 ± 0.2 y	2.9 ± 0.2 x	3.4 ± 0.2 x	2.0 ± 0.2 w	<0.001
18:1(n-7)	4.5 ± 0.1 z	3.1 ± 0.1 y	1.5 ± 0.1 w	2.6 ± 0.1 x	1.4 ± 0.1 w	<0.001
18:1(n-9)	21.1 ± 1.4 w	27.8 ± 1.4 x	34.8 ± 1.5 y	36.0 ± 1.4 y	48.3 ± 1.4 z	<0.001
20:1(n-9)	2.1 ± 0.1 zyx	2.0 ± 0.1 yx	1.8 ± 0.1 x	2.4 ± 0.1 z	2.2 ± 0.1 zy	0.012
Total MUFA[b]	33.8 ± 1.3 w	37.7 ± 1.3 x	41.3 ± 1.4 yx	44.8 ± 1.3 y	54.4 ±1.3 z	<0.001
18:2(n-6)	6.3 ± 0.5	7.9 ± 0.5	7.4 ± 0.5	7.6 ± 0.5	8.2 ± 0.5	0.106
20:4(n-6)	1.5 ± 0.0 z	1.1 ± 0.0 y	0.4 ± 0.0 x	1.0 ± 0.0 y	0.4 ± 0.0 x	<0.001
All (n-6)[c]	9.5 ± 0.6	10.8 ± 0.6	9.6 ± 0.6	10.1 ± 0.6	10.1 ± 0.6	0.521
20:5(n-3)	9.3 ± 0.2 z	6.5 ± 0.2 y	1.3 ± 0.2 w	5.3 ± 0.2 x	1.4 ± 0.2 w	<0.001
22:5(n-3)	3.9 ± 0.2 z	2.6 ± 0.2 y	0.3 ± 0.2 w	1.8 ± 0.2 x	0.4 ± 0.2 w	<0.001
22:6(n-3)	17.2 ± 0.6 z	8.6 ± 0.6 y	1.5 ± 0.7 x	8.1 ± 0.6 y	2.1 ± 0.6 x	<0.001
All (n-3)	31.4 ± 1.0 z	18.4 ± 1.0 y	3.3 ± 1.0 x	15.8 ± 1.0 y	4.0 ± 1.0 x	<0.001
Total PUFA[d]	43.0 ± 1.6 z	30.9 ± 1.6 y	13.6 ± 1.7 x	27.2 ± 1.6 y	14.7 ± 1.6 x	<0.001
Total LC-PUFA[e]	33.1 ± 1.0 z	19.7 ± 1.0 y	3.9 ± 1.0 x	17.0 ± 1.0 y	4.6 ± 1.0 x	<0.001
Total MC-PUFA[f]	8.9 ± 0.6	10.2 ± 0.6	9.0 ± 0.6	9.3 ± 0.6	9.5 ± 0.6	0.576
(n-3):(n-6)	3.3 ± 0.0 z	1.7 ± 0.0 y	0.3 ± 0.0 x	1.6 ± 0.0 y	0.4 ± 0.0 x	<0.001
Total lipid (% wet tissue)	15.7 ± 1.4 w	18.5 ± 1.4 xw	24.7 ± 1.6 zy	20.6 ± 1.4 yx	27.0 ± 1.4 z	<0.001

[a] Includes 10:0, 15:0, and 17:0 in addition to the individually reported SFA.

[b] Includes 14:1, 17:1, and 24:1(n-9) in addition to the individually reported MUFA.

[c] Includes 18:3(n-6), 20:2(n-6), and 20:3(n-6) in addition to the individually reported (n-6) fatty acids.

[d] Includes 16:2(n-4), 16:3(n-4), 18:3(n-4), 20:4(n-3), 20:2(n-6), and 20:3(n-6) in addition to the individually reported PUFA.

[e] Includes 20:3(n-6) in addition to the individually reported LC-PUFA.

[f] Includes 18:3(n-4) and 18:3(n-6) in addition to the individually reported MC-PUFA.

passed (Trushenski et al. 2008a). In terms of SFA content, the 8-week fillets from the present study are most comparable to the 28-week fillets from the previous study, but are markedly lower in terms of LC-PUFA content (19.7% versus 29.2%). Total fillet LC-PUFA levels in the 100%-FO control group were also reduced in the present study compared with the previous trial (28.2% versus 34%). The primary difference between these two trials is the starting size of the fish and their growth rate during the feeding period. The previous trial began with 50-g fish that demonstrated a specific growth rate (SGR) of 1.4%/d during the first 20 weeks and 0.5%/d during the last 8 weeks, or 1.2%/d over the course of the 28-week trial (values calculated from mean performance of 50%-CO group only, not pooled data). In the present study, fish in the 50%-CO group started at an average individual mass of approximately 26 g, and gained 2.7%/d during the 8-week culture period. The two-fold increase in growth rate explains the rapid divergence in FA profile achieved in the present work, but does little to explain the different rates observed in the two trials with respect to the loss of LC-PUFA within the total lipid profile.

We have generated a considerable amount of data that suggests selective FA metabolism is a major determinant of tissue FA profile in sunshine bass (Lane et al. 2006; Trushenski et al. 2008a); however, incorporation or exclusion of FAs varies among lipid classes (Trushenski et al. 2008b). The paradigm of selective FA metabolism invokes selective incorporation of certain FAs during new tissue synthesis or lipid accumulation, via remodeling of existing lipid fractions, or some combination thereof. Growth can occur via hyperplastic (increases in total number of cells) or hypertrophic (increases in individual cell size) processes. In fishes, muscular hypertrophy is associated with rapid weight gain, whereas muscular hyperplasia is associated with slower growth (Dumas et al. 2007). Although both processes are occurring to some extent, juvenile growth in fishes is generally dominated by hypertrophic growth (Koumans and Akster 1995). By their nature, muscular hypertrophy and hyperplasia are associated with greater synthesis and accumulation of neutral (i.e., triacylglyceride [TAG]) and polar (i.e., phospholipid [PL]) lipids, respectively. Given the differences in FA selectivity and plasticity within these lipid classes (Trushenski et al., in press), one might expect a FA profile change during rapid (hypertrophic)

TABLE 5.—Total intraperitoneal fat fatty acid composition in sunshine bass. See Table 3 for additional details.

Fatty acids	100% Fish oil	50% Coconut oil	100% Coconut oil	50% Palm oil	100% Palm oil	P-value
12:0	0.3 ± 0.4 x	14.0 ± 0.4 y	23.2 ± 0.4 z	0.1 ± 0.4 x	0.2 ± 0.4 x	<0.001
14:0	7.8 ± 0.2 x	11.7 ± 0.2 y	14.6 ± 0.2 z	4.6 ± 0.2 w	2.1 ± 0.2 v	<0.001
16:0	19.6 ± 0.2 x	16.6 ± 0.2 w	15.9 ± 0.2 v	22.8 ± 0.2 y	25.8 ± 0.2 z	<0.001
18:0	2.9 ± 0.1 x	2.6 ± 0.1 w	2.9 ± 0.1 x	3.2 ± 0.1 y	3.8 ± 0.1 z	<0.001
Total SFA[a]	30.9 ± 0.6 x	45.7 ± 0.5 y	57.8 ± 0.6 z	31.0 ± 0.5 x	32.1 ± 0.5 x	<0.001
16:1(n-7)	12.2 ± 0.1 z	7.6 ± 0.1 y	3.8 ± 0.1 x	7.5 ± 0.1 y	3.1 ± 0.1 w	<0.001
18:1(n-7)	3.7 ± 0.0 z	2.4 ± 0.0 x	1.4 ± 0.0 v	2.5 ± 0.0 y	1.5 ± 0.0 w	<0.001
18:1(n-9)	15.0 ± 0.5 v	16.4 ± 0.5 w	20.4 ± 0.5 x	29.3 ± 0.5 y	41.3 ± 0.5 z	<0.001
20:1(n-9)	1.3 ± 0.0 z	1.0 ± 0.0 y	1.0 ± 0.0 y	1.3 ± 0.0 z	1.3 ± 0.0 z	<0.001
Total MUFA[b]	32.4 ± 0.5 x	27.7 ± 0.5 w	26.7 ± 0.5 w	40.8 ± 0.5 y	47.3 ± 0.5 z	<0.001
18:2(n-6)	10.2 ± 0.2 w	10.8 ± 0.1 x	9.9 ± 0.2 w	13.7 ± 0.1 y	15.8 ± 0.1 z	<0.001
20:4(n-6)	1.1 ± .0 z	0.6 ± 0.0 y	0.3 ± 0.0 x	0.6 ± 0.0 y	0.2 ± 0.0 w	<0.001
All (n-6)[c]	12.6 ± 0.2 x	12.6 ± 0.1 x	11.5 ± 0.2 w	15.4 ± 0.1 y	16.9 ± 0.2 z	<0.001
18:3(n-3)	1.7 ± 0.0 z	1.3 ± 0.0 y	0.9 ± 0.0 x	1.3 ± 0.0 y	0.9 ± 0.0 x	<0.001
18:4(n-3)	1.7 ± 0.0 z	1.0 ± 0.0 y	0.3 ± 0.0 w	0.9 ± 0.0 x	0.2 ± 0.0 v	<0.001
20:4(n-3)	1.2 ± 0.0 z	0.8 ± 0.0 y	0.2 ± 0.0 w	0.7 ± 0.0 x	0.2 ± 0.0 w	<0.001
20:5(n-3)	9.8 ± 0.2 z	5.2 ± 0.1 y	1.2 ± 0.2 x	4.9 ± 0.1 y	1.1 ± 0.1 x	<0.001
22:5(n-3)	2.8 ± 0.0 z	1.8 ± 0.0 y	0.4 ± 0.0 w	1.5 ± 0.0 x	0.4 ± 0.0 w	<0.001
22:6(n-3)	6.4 ± 0.1 z	3.4 ± 0.1 y	0.8 ± 0.1 x	3.2 ± 0.1 y	0.8 ± 0.1 x	<0.001
All (n-3)	20.3 ± 0.3 z	11.3 ± 0.3 y	2.7 ± 0.3 w	10.4 ± 0.3 x	2.4 ± 0.3 w	<0.001
Total PUFA[d]	36.8 ± 0.4 z	26.6 ± 0.4 x	15.5 ± 0.4 v	28.3 ± 0.4 y	20.6 ± 0.4 w	<0.001
Total LC-PUFA[e]	21.7 ± 0.3 z	12.1 ± 0.3 y	3.1 ± 0.3 w	11.1 ± 0.3 x	2.7 ± 0.3 w	<0.001
Total MC-PUFA[f]	14.4 ± 0.2 x	13.8 ± 0.2 w	11.6 ± 0.2 v	16.4 ± 0.2 y	17.3 ± 0.2 z	<0.001
(n-3):(n-6)	1.6 ± 0.0 z	0.9 ± 0.0 y	0.2 ± 0.0 w	0.7 ± 0.0 x	0.1 ± 0.0 v	<0.001

[a] Includes 10:0, 15:0, and 17:0 in addition to the individually reported SFA.
[b] Includes 14:1, 17:1, and 24:1(n-9) in addition to the individually reported MUFA.
[c] Includes 18:3(n-6), 20:2(n-6), and 20:3(n-6) in addition to the individually reported (n-6) fatty acids.
[d] Includes 16:2(n-4), 16:3(n-4), 18:3(n-4), 20:4(n-3), 20:2(n-6), and 20:3(n-6) in addition to the individually reported PUFA.
[e] Includes 20:3(n-6) in addition to the individually reported LC-PUFA.
[f] Includes 18:3(n-4) and 18:3(n-6) in addition to the individually reported MC-PUFA.
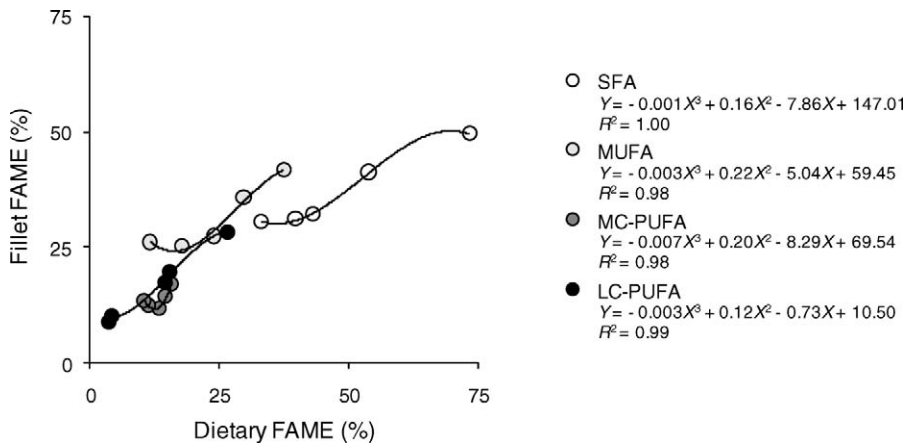

FIGURE 1.—Relationships between dietary and fillet fatty acid composition in sunshine bass (expressed as the relative percentages of fatty acid methyl esters [FAMEs]) for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), medium-chain polyunsaturated fatty acids (MC-PUFA), and long-chain polyunsaturated fatty acids (LC-PUFA). The data points are the mean compositional values reported in Tables 2 and 3; the trend lines represent third-order polynomial regressions fitted to the mean values.
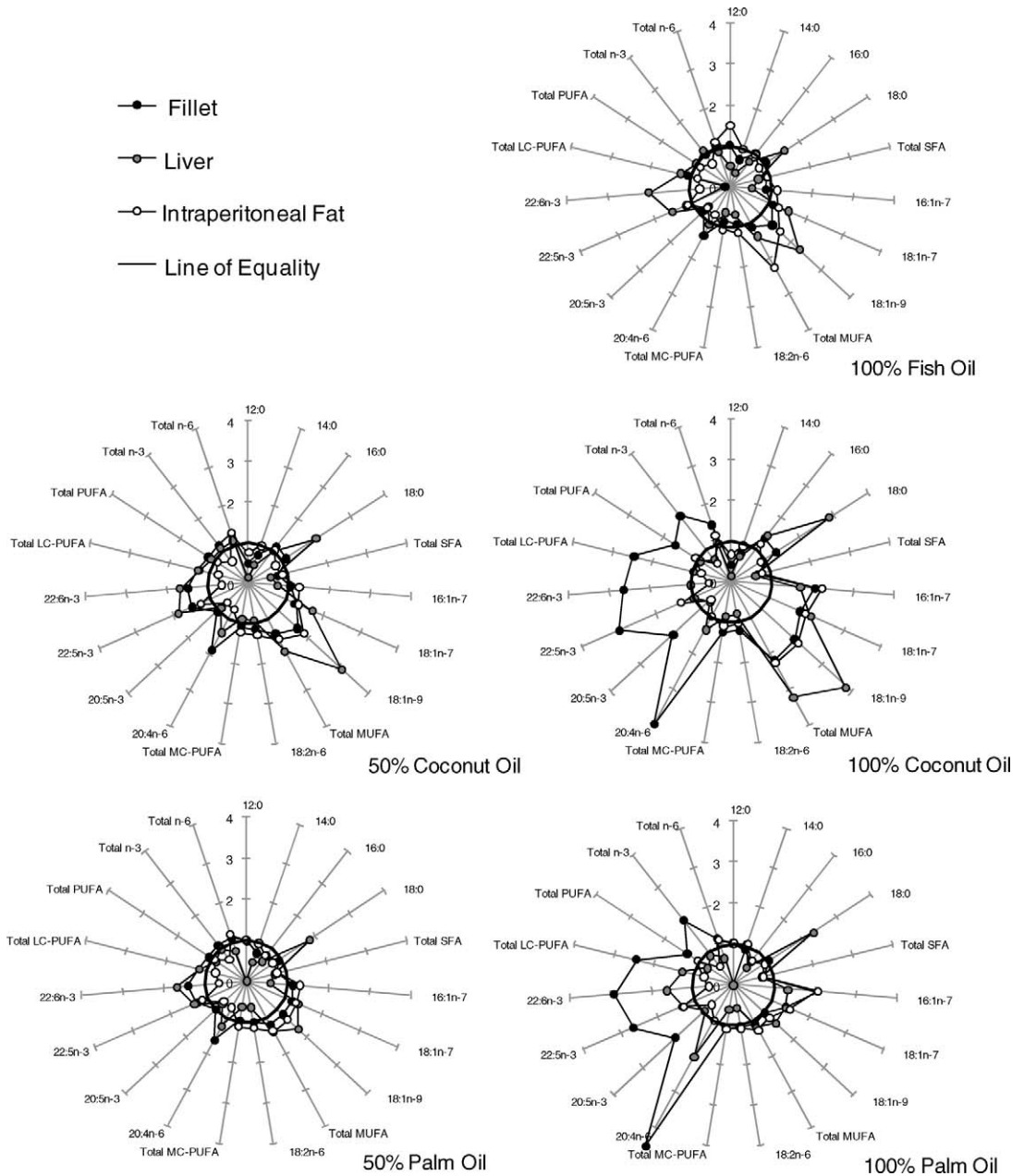
FIGURE 2.—Fatty acid composition of the tissue total lipid of sunshine bass expressed as a fraction of the dietary fatty acid composition (a value of 1 represents equality between the tissue and dietary composition).

growth to occur primarily via dilution processes (accumulation of TAG), whereas a FA profile change during slower (hyperplastic growth) may be largely determined by selective processes (synthesis and remodeling of PL). Although there is no data in the literature that directly support this hypothesis, some anecdotal information appears to suggest this may be

the case. Haugen et al. (2006) monitored fillet FA profile change of Atlantic halibut *Hippoglossus hippoglossus* fed marine-derived or soy-derived oils and cultured under constant temperature and light (faster growth rate) or ambient seasonal conditions (slower growth rate). Although those authors did not address growth rate as a covarying or contributing factor, the

TABLE 6.—Production performance of sunshine bass by dietary treatment group. Within rows, means followed by different letters are significantly different.

Production variable	100% Fish oil	50% Coconut oil	100% Coconut oil	50% Palm oil	100% Palm oil	P-value
Initial weight (g)[a]	25.4 ± 0.3	26.0 ± 0.3	25.0 ± 0.3	25.0 ± 0.3	25.4 ± 0.3	0.198
Final weight (g)[b]	127 ± 7 z	120 ± 7 z	96 ± 8 y	127 ± 7 z	114 ± 7 zy	0.038
Weight gain (%)[bc]	402 ± 28 z	362 ± 28 z	284 ± 31 y	408 ± 28 z	349 ± 28 zy	0.049
Feed intake[ad]	3.5 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	3.7 ± 0.2	4.2 ± 0.2	0.103
Feed conversion ratio[be]	1.1 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	0.215
Specific growth rate[bf]	2.8 ± 0.1	2.7 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	0.052
Hepatosomatic index[bg]	1.4 ± 0.1 y	1.6 ± 0.1 y	2.4 ± 0.1 z	1.6 ± 0.1 y	2.1 ± 0.1 z	<0.001

[a] Values represent least-squares means $\pm$ SEs of average individual performance criteria of five or more replicate tanks.
[b] Values represent least-squares means $\pm$ SEs of duplicate individuals from five or more replicate tanks.
[c] [(final weight $-$ initial weight)/initial weight] $\times$ 100.
[d] [total dry matter intake/(initial weight $\times$ final weight)$^{0.5}$/number of days fed] $\times$ 100.
[e] Consumption/weight gain.
[f] [$\log_{10}$(final weight) $-$ $\log_{10}$(initial weight)]/d.
[g] (liver mass/whole-body mass) $\times$ 100.

mean fillet composition data seem to suggest that 18:2(n-6) content (FA composition of the two experimental feeds differed most greatly with respect to 18:2[n-6] content) changed more rapidly among slow-growing fish within the PL fraction, whereas among fast-growing fish, the rate of change was greater within the TAG fraction. Again, this suggests that overall fillet FA profile of fast-growing fish is more strongly influenced by accumulation of neutral lipid and, therefore, less subject to the processes of selective FA metabolism. This hypothesis would explain the rapid FA profile change we observed in the present work relative to our previous study; however, additional research is needed to verify the influence of growth rate on FA profile change in fishes.

Although fillet FA profile divergence in the present work may have been largely driven by nonselective processes due to rapid growth, there appears to have been some distinction between FA classes in terms of fillet deposition. As our laboratory has previously demonstrated (Trushenski et al. 2008a), LC-PUFA were disproportionately enriched in fillets of sunshine bass fed the reduced or FO-free feeds (Figure 2). A direct linear relationship between dietary FA composition and fillet FA composition is often reported as an indicator of FA dilution. In the present study, third-order polynomial regressions were much more representative of the feed–fillet relationships than simple linear regressions (R^2 values ranged from 0.98 to 1.00, Figure 1). For SFA, MUFA, and LC-PUFA, there appeared to be a threshold of dietary influence (i.e., the fillet FA content mirrored the dietary content up to a certain point, beyond which increasing dietary levels did not result in commensurate changes in the fillet). A similar threshold was not observed for MC-PUFA; however, our feeds did not vary considerably in MC-PUFA content, and it is likely that the range of dietary

levels was simply insufficient to identify a breakpoint in the feed–fillet relationship for this FA class. Nonetheless, it is interesting to note the slope of the linear segment of the regressions appears to be greatest for MC-PUFA, suggesting these FAs may be able to "outcompete" other FAs for deposition in fish tissues (Bell et al. 1996; Hvattum et al. 2000; Trushenski et al. 2008a, 2008b).

Palm oil is well utilized by a wide variety of aquatic species as an alternative to fish oil (Al-Owafeir and Belal 1996; Ng et al. 2000; Lim et al. 2001; Bell et al. 2002; Ng 2002; Ng et al. 2003, 2004, 2007; Fonseca-Madrigal et al. 2005; Bahurmiz and Ng 2007). Reductions in consumption and growth performance have not been routinely reported for fish raised on feeds based on palm oil (Ng 2002), although a decreased digestibility of SFA has been noted at cooler culture temperatures and higher dietary palm-oil inclusion rates (Ng et al. 2004, 2007). Coconut oil has not been as widely researched in aquaculture nutrition, but has been used successfully in feeding aquatic livestock (Alava 1998; Fontagné et al. 1999) and, based on its FA composition, should be readily digested and absorbed (Olsen et al. 1998). Craig and Gatlin (1995) noted that red drum Sciaenops ocellatus were quite effective in utilizing CO as a dietary lipid source, but only if minimum essential fatty acid (EFA) requirements were met. Complete replacement of FO with CO (in feeds based on solvent-extracted fish meal) significantly reduced weight gain; however, when CO-based feeds were supplemented with 2% FO or 1% (n-3) HUFA blend, production performance was either unaffected or improved (Craig and Gatlin 1995). It is possible that our feeds, based on 20% full-fat fish meal, were insufficient to meet the minimum dietary requirements of sunshine bass. Using the same basal formulation, Lewis and Kohler (2008) noted reduced

growth in sunshine bass when more than 60% of dietary FO was replaced with canola oil, and suggested EFA content as a limiting factor. However, one would expect the 100% PO to have yielded similar results if an EFA deficiency were solely to blame. It is perhaps most likely that the impaired performance observed in the 100%-CO group was a consequence of increased metabolic costs or PL deficiency.

Although SFA appear to be adequate, perhaps preferred, substrates for energy production in fishes (Henderson and Sargent 1985; Kiessling and Kiessling 1993; Henderson 1996; Froyland et al. 2000), provision of SFA also induces increased elongation and desaturation activity (Bell et al. 2002). Growth was reduced among channel catfish *Ictalurus punctatus* fed CO, but excellent growth was observed among those fed beef tallow (Stickney and Andrews 1972). Those authors concluded medium-chain SFA and MUFA (beef tallow) are effectively utilized by this species, whereas shorter-chain SFA (CO) are not; however, total carcass lipid did not vary between these groups, suggesting equivalent uptake of either lipid source. It is perhaps more likely that shorter-chain SFA were being biotransformed, incurring additional metabolic costs, and reducing the total availability of energy for somatic growth. Supporting this hypothesis, the predominant FAs in the CO feed, 12:0 and 14:0, were not commensurately represented within the liver lipid (primary site of FA biosynthesis and transformation), whereas 16:0, 18:0, and 18:1(n-9) were enriched relative to the diet (Stickney and Andrews 1972). Although reductions in growth performance were not observed, Caballero et al. (2002) reported similar increases in elongation–desaturation products in livers of rainbow trout *Oncorhynchus mykiss* fed diets containing increased levels of SFA and MUFA. In the present study, the 100%-FO treatment and both 50%-FO replacement treatments exhibited tissue FA profiles largely representative of dietary composition; however, complete replacement of FO resulted in considerable deviations from dietary profile. Within the livers of fish fed the 100%-CO and 100%-PO feeds, 18:0 and 18:1(n-9) were enriched relative to the diet whereas 12:0, 14:0, and 16:0 were notably reduced or absent (Figure 2). Again, these data seem to suggest elongation and desaturation of surplus dietary SFA within the hepatic tissue. Although sunshine bass are generally considered incapable of synthesizing LC-PUFA in meaningful amounts, as vertebrates, they are undoubtedly able to produce longer-chain SFA and 18:1(n-9) from their precursors. However, additional research is needed to confirm increased FA biotransformation among sunshine bass fed SFA-rich feeds and to address whether this may affect energy balance and growth.

Depending on the extraction process used, PO contains 20–2,000 µg/L PL, whereas the polar lipid content of CO is negligible (Gunstone et al. 1994). As noted by Tocher (2003), many freshwater fish species lack the ability to synthesis PL. Give the limited biosynthetic capacity of sunshine bass with respect to FA biotransformation, it is reasonable to assume that this hybrid species is also unable to meet PL requirements endogenously. Although Sealey et al. (2001) demonstrated growth performance of sunshine bass was unaffected by dietary supplementation with soy lecithin (as a source of phosphatidylcholine), the basal diet contained approximately 7%-PL-rich FO, and was probably sufficient to meet the PL requirement of these fish without the addition of lecithin. Although the PL requirement may be lower among juvenile and subadult individuals compared with larval fish (Tocher et al. 2008), sunshine bass undoubtedly exhibit some nominal requirement for PL throughout their life cycle. Unfortunately, few studies have evaluated the PL requirements of fish beyond the early juvenile stages, and none have addressed this question in the context of sunshine bass nutrition. Although some alternative lipid sources provide considerable amounts of PL (e.g., soybean- and canola-derived feedstuffs), others may be limited or wholly deficient with respect to this nutrient (Tocher et al. 2008). Additional research is needed to establish the PL requirement of sunshine bass (and other species) at the juvenile and subadult stages to assess the potential for PL deficiency in alternative lipid-based aquafeeds.

Based on the production performance and tissue FA profiles observed in the present study, CO and PO appear to have promise as alternatives to FO in feeds for sunshine bass. However, the extent to which these alternative lipids can be implemented in aquafeeds will depend on the ability of nutritionists to resolve issues of digestibility and utilization associated with these feedstuffs and to determine the effect FO replacement may have on bioenergetics of cultured fishes.

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