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Evaluation of the Impact of Dietary Petroselinic Acid on the Growth Performance, Fatty Acid Composition, and Efficacy of Long Chain-Polyunsaturated Fatty Acid Biosynthesis of Farmed Nile Tilapia

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ABSTRACT: The present study aimed to investigate the potential role of dietary petroselinic acid (PSA) in enhancing the n-3 long-chain polyunsaturated fatty acid (LC-PUFA) content in fish tissues. Three isolipidic casein-based diets were formulated to comprise graded levels of PSA (0, 10, or 20% of total fatty acid) with the incremented inclusion of coriander seed oil. Fish growth and nutrient digestibility were not significantly ($P > 0.05$) influenced by dietary PSA level. In general, dietary PSA affected the fatty acid composition of tilapia tissues and whole-body, which reflected dietary fatty acid ratios. Dietary PSA significantly ($P < 0.05$) increased β-oxidation, particularly on α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6). This study provided evidence that PSA, a pseudoproduct mimicking the structure of 18:3n-6, did reduce Δ-6 desaturation on 18:2n-6 but, contrary to popular speculation, did not stimulate more Δ-6 desaturase activity on 18:3n-3. The overall Δ-6 desaturase enzyme activity may be suppressed at high dietary levels of PSA. Nevertheless, the n-3 and n-6 LC-PUFA biosynthesis was not significantly inhibited by dietary PSA, indicating that the bioconversion efficiency is not modulated only by Δ-6 desaturase. The deposition of n-3 LC-PUFA in liver and fillet lipids was higher in fish fed PSA-supplemented diets.

KEYWORDS: *petroselinic acid, fish oil, tilapia, fatty acid metabolism, coriander seed oil*

INTRODUCTION

Aquaculture is rapidly growing, with a global production of 59.9 million metric tons valued at U.S. $119.4 billion in 2010.[1] The rapid expansion of the aquaculture industry is partly driven by the wide use of aquafeeds, which traditionally use fish oil (FO) as the major dietary lipid source. It is estimated that a total of 764 000 metric tons of FO was used by the aquaculture industry for aquafeed production[2] in 2010, which constituted about 86.8% of global supply.[3] Undeniably, FO is becoming increasingly costly, and identifying alternatives is increasingly being investigated.

Among all of the alternative lipid sources, vegetable oils (VO) appear to be predominant candidates as they are more readily available, cost-effective, sustainable, and environmentally friendly.[4] Partial or full replacement of FO by various VO in fish diets displayed no detrimental effect on growth performance and feed efficiency when the essential fatty acid requirements of fish were met.[5,6] Dietary fatty acids have been established to modulate fatty acid metabolism[7−9] and also to affect the fatty acid composition in fish tissue.[5,7,10,11] Given that VO lacks long-chain polyunsaturated fatty acids (LC-PUFA), the most stringent drawback of FO replacement in aquafeeds is the decrease in the health-beneficial n-3 LC-PUFA content of fish fillet, which compromises the nutritional quality of the fish for human consumers.[4] Therefore, it is important that alternative lipid sources with the potential to improve the n-3 LC-PUFA content of fish be evaluated in the diets of farmed fish.

Tilapia are capable of transforming the essential fatty acids α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) into LC-PUFA [eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-6)] via a series of elongation and desaturation steps.[12] Additionally, we have previously shown that tilapia farming can be a net producer of n-3 LC-PUFA despite the tilapia being fed LC-PUFA-deprived diets. Moreover, it has been demonstrated that Nile tilapia of the genetically improved farmed tilapia (GIFT) strain has a greater ability for fatty acid neogenesis, elongation, and Δ-5 and Δ-6 desaturation than red hybrid tilapia.[13]

Δ-6 Desaturase is known as the rate-limiting enzyme because it is involved in the first step of biosynthesizing LC-PUFA by desaturating the C_{18} PUFA precursors, 18:3n-3 and 18:2n-6, to stearidonic acid (18:4n-3) and γ-linolenic acid (18:3n-6), respectively.[14] Many studies have reported that the efficiency and affinity of the Δ-6 desaturase is dependent on the availability of the substrate enzyme,[15,16] as well as competition between 18:3n-3 and 18:2n-6 for the Δ-6 desaturase enzyme.[17] Meanwhile, dietary 18:3n-6 and 18:4n-3 have been described to bypass the primary rate-limiting step and thus the subsequent desaturation and elongation are dependent upon the dietary levels of the substrate 18:3n-6 and 18:4n-3; however, the biosynthesis derived from these fatty acid precursors was insufficient to compensate for LC-PUFA deficiencies.[18,19]

Petroselinic acid (PSA, *cis*-6-octadecenote, 18:1n-12) is a monounsaturated fatty acid (MUFA) and is a major fatty acid of coriander seed oil. Studies on rats reported that PSA inhibited the Δ-6 desaturase activity on 18:2n-6 with its Δ6-*cis*-double bond, a pseudoproduct mimicking the structure of 18:3n-6 and which then subsequently reduced the 20:4n-6 content in tissue lipids.[20−22] It is speculated that reduced Δ-6 desaturation on 18:2n-6 will stimulate more Δ-6 desaturase

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activity on 18:3n-3 and may ultimately enhance the synthesis and bioaccumulation of more health-promoting n-3 LC-PUFA content in fish fillets. In light of the lack of comparative studies on the precise mechanism underpinning the feedback inhibition of Δ-6 desaturated fatty acids, there is considerable interest to investigate the effects of PSA on the nutritional regulation of fatty acid metabolism in fish. Therefore, the aim of the present study was to evaluate the effects of PSA in LC-PUFA deprived diets on the overall in vivo enzyme activities involved in the fatty acid metabolism of GIFT tilapia. The impact of dietary PSA on growth, body proximate composition, and tissue fatty acid composition was examined, with a specific focus on the endogenously synthesized LC-PUFA content in tilapia.

MATERIALS AND METHODS

Experimental Diets. Three semipurified experimental diets were formulated to be isonitrogenous and isoenergetic. The ingredient composition of the diets were the same, with the exception of the added lipid sources, which were added at a level of 10% of the total diet (Table 1). The lipid sources were formulated to contain similar major fatty acid classes, a constant ratio of 18:3n-3 to 18:2n-6, and a graded inclusion of PSA by blending different proportions of sunflower oil (21.8–24.7%), linseed oil (37–40.9%), canola oil (0.9–41.2%), and coriander seed oil (0–33.5%). All oils (except coriander seed oil) and coriander seeds were purchased from grocery stores. Coriander seed oil was extracted using chloroform and methanol,[23] with 0.01% butylated hydroxytoluene added during the extraction process to prevent lipid oxidation. The diets were designated 0% PSA, 10% PSA, or 20% PSA on the basis of the formulated PSA content of total dietary fatty acids. Casein and gelatin were used as the main sources of protein in diets, whereas dextrin was included as the carbohydrate source. Chromic oxide was included in diet at an inclusion rate of 0.5% as an inert marker for the determination of apparent nutrient digestibility. All purified feed ingredients were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dry ingredients were mixed homogenously in a Hobart mixer. Then, the oil was thoroughly mixed with the ingredient mixture, and distilled water was added. The moist dough was screw-pressed through a 2 mm die, and the feed pellets formed were fan-dried and stored frozen at −20 °C until use.

Table 1. Ingredient and Proximate Composition (g/kg Diet) of Experimental Diets

	experimental diets[a]		
	0% PSA	10% PSA	20% PSA
ingredient			
casein	330.0	330.0	330.0
gelatin	60.0	60.0	60.0
sunflower oil	21.8	23.3	24.7
canola oil	41.2	18.4	0.9
linseed oil	37.0	39.4	40.9
coriander seed oil		18.9	33.5
dextrin	268.2	268.2	268.2
vitamin mix[b]	30.0	30.0	30.0
mineral mix[c]	40.0	40.0	40.0
dicalcium phosphate	10.0	10.0	10.0
chromic oxide	5.0	5.0	5.0
carboxymethyl cellulose	15.0	15.0	15.0
α-cellulose	141.8	141.8	141.8
proximate composition			
dry matter	907.3	906.5	907.6
protein	368.5	364.8	361.8
lipid	88.4	90.7	87.6
ash	49.2	49.7	49.4
NFE[d]	448.1	465.3	463.5
energy[e] (kJ/g)	19.9	20.2	20.0

[a]Petroselinic acid (PSA), % total dietary fatty acids. [b]Vitamin mix (g/kg premix): ascorbic acid, 45.00; inositol, 5.00; choline bitartrate, 136.06; niacin, 4.50; riboflavin, 1.00; pyridoxine·HCl, 1.00; thiamin·HCl, 0.92; *d*-calcium panthothenate, 3.00; retinyl acetate, 0.60; cholecalciferol, 0.083; menadione 1.67; DL-α-tocopheryl acetate (250 IU/g), 8; *d*-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135; cellulose, 783.167. [c]Mineral mix (g/kg premix): calcium phosphate monobasic, 135.49; calcium L-lactate hydrate, 327.00; ferric citrate, 29.70; magnesium sulfate·7H_2O, 132.00; potassium phosphate dibasic, 239.80; sodium phosphate monobasic·H_2O, 87.20; sodium chloride, 43.50; potassium iodide, 0.15; cuprous chloride, 0.20; manganous sulfate·H_2O, 0.80; cobalt chloride·6H_2O, 1.00; zinc sulfate·7H_2O, 3.00; sodium selenite, 0.011. [d]NFE, nitrogen-free extract, calculated by difference. [e]Energy, calculated on the basis of 23.6 kJ/g protein, 39.5 kJ/g lipid, and 17.2 kJ/g carbohydrate.

Fish and Experimental Design. GIFT tilapia (*Oreochromis niloticus*) fingerlings were obtained from the Aquaculture Extension Centre, Jitra, Malaysia. All fish were acclimated to the experimental system for 2 weeks before the start of the feeding trial. Fish were fed twice daily with a conditioning diet with protein and energy contents similar to those of the experimental diets but containing no added lipid prior to commencement of the experiment. Groups of 15 fish (mean weight ± SD, 4.4 ± 0.0 g) were randomly selected, weighed, and stocked into 12 aquaria. Each experimental diet was fed to four replicate groups of tilapia. The flow-through aquaria system setup and water quality parameters were as previously described by Ng et al.[24] Fish were hand-fed twice a day (9:00 a.m., 5:00 p.m.) to apparent satiation for 59 days, and feed consumption was recorded daily. Fish were batch-weighed weekly.

Sample Collection. At the beginning of the experiment, an initial sample of 20 fish was culled for whole-body proximate and fatty acid analysis. Another 20 fish were killed and filleted, and liver tissue was excised and kept frozen for subsequent proximate composition and fatty acid analysis. Fish were starved for 48 h prior to sampling. After 4 weeks into the feeding trial, fecal material (intact strands) was siphoned and collected with a fine mesh net. Fecal samples collected from each aquarium were pooled, freeze-dried, and finely ground before analysis. At the end of the experiment, all fish were individually weighed and their total length measured. A sample of six fish per tank was randomly sampled and euthanized, and blood was collected into heparinized tubes. The tubes were then microcentrifuged to determine the percent hematocrit value. The sampled fish were dissected to remove various tissues for analysis and then skinned and filleted. Liver, viscera, gonads, and intraperitoneal fat were excised and weighed for the determination of hepatosomatic index (HSI), viscerosomatic index (VSI), gonadosomatic index (GSI), and intraperitoneal fat index, respectively. These body indices were calculated as a percentage of organ or tissue to the whole-body weight of individual fish.[25,26] Liver and fillet samples were pooled and kept frozen for subsequent chemical analysis. The remaining fish from each aquarium were culled and stored frozen at −20 °C for subsequent whole-body composition analysis.

Chemical Analysis. Proximate analysis of diet ingredients, experimental diets, feces, and fish whole-body samples was conducted using standard AOAC methods.[27] The chromic oxide content of diets and feces was determined according to the wet-acid digestion method of Furukawa and Tsukahara.[28] All samples were freeze-dried to constant weight and finely ground before lipid extraction. Total lipid was extracted from samples using a modified Folch et al.[23] protocol. Weighed samples were soaked overnight in chloroform/methanol/water containing potassium chloride in a separatory funnel. After separation, total lipid content of samples was determined gravimetrically after evaporation of solvents using a rotary evaporator. Fatty acids were esterified into methyl esters which were then resolved and analyzed by a gas chromatograph (Shimadzu GC-2010) equipped with a flame ionization detector and Shimadzu GC solution software as previously described.[13]

Calculations and Statistical Analysis. The apparent digestibility coefficients (ADC) of fatty acids were calculated from a standard formula.[29] The computation of the whole-body fatty acid balance was carried out as initially described by Turchini et al.[30] and with further developments as described in Turchini et al.[31] and Turchini and Francis[8] to assess the fatty acid metabolism of the tilapia. All data were subjected to a one-way analysis of variance (ANOVA) using SPSS 11.5 (SPSS Inc., Chicago, IL, USA) to determine if significant differences occurred among dietary treatments. When a significant treatment effect was observed, a Duncan's multiple-range test was used to compare means. Treatment effects were considered at the $P < 0.05$ level of significance.

RESULTS

Diet Composition. The three semipurified experimental diets were isonitrogenous and isolipidic (Table 1), and their fatty acid composition reflected the fatty acid composition of the dietary lipid source (Table 2). The major fatty acid classes of the three diets were similar and comprised on average of 9% saturated fatty acids (SFA), 41% MUFA, and 50% PUFA. The dietary PUFA content was dominated by 18:3n-3 and 18:2n-6 and a minimal amount of 18:3n-6 (only in the 0% PSA diet). The 18:3n-3/18:2n-6 ratio was close to 1 for all diets. Concomitant with the increasing dietary level of PSA from 0 to 20%, the dietary oleic acid (18:1n-9) relative levels decreased from 38 to 20% (Table 2).

Growth Performance, Nutrient Digestibility, and Whole-Body Proximate Composition. After 59 days of the feeding trial, percentage weight gained by tilapia was about 800% in all treatments, and there was no significant difference ($P > 0.05$) in final weight, which ranged from 39.0 ± 1.2 to 39.6 ± 3.0 g (Table 3). Similarly, the specific growth rate was almost identical in all treatments. The survival rate of fish was high, ranging from 95.6 to 97.8%. No significant differences were noted in feed utilization and biometric parameters. The apparent digestibility coefficients of dry matter ($ADC_{dry\ matter}$) and lipid (ADC_{lipid}) were unaffected by the dietary treatment, ranging from 62.0 to 70.7% and from 91.0 to 94.0%, respectively (Table 3). ADC of individual fatty acids showed significant differences only for 14:0, 18:2n-6, and 18:3n-3 (data not shown). Fish fed the 20% PSA diet had the highest moisture content, which was significantly higher ($P < 0.05$) than those fed the 0% PSA diet (Table 4). However, whole-body protein, lipid, and ash contents of fish were not significantly affected by the dietary treatments.

Fatty Acid Composition of Fish Tissue and Whole Body. The fatty acid composition of the fillets and livers of fish fed diets with increasing PSA is shown in Tables 5 and 6, respectively. In the fillet, 18:2n-6 and 18:3n-3 were significantly higher in fish fed the 0% PSA diet than in those fed the PSA-added diets. Relative percentage depositions of 5.2 and 9.8% PSA were recorded in the fillet lipids of fish fed the 10 and 20% PSA diets, respectively. Fish fed the 20% PSA diet showed the highest content of docosapentaenoic acid (DPA, 22:5n-3) at 1.05%, which was significantly higher compared to the DPA found in the fillet lipids of fish fed the 0% PSA diet. Meanwhile, the total n-6 PUFA was lowest in fish fed dietary 20% PSA (16.2%), which was significantly lower than those fed the 0% PSA diet (17.5%). The relative EPA+DHA content was higher in fillet lipids of fish fed diets containing PSA than those fed the 0% PSA diet, although no significant differences were detected. The major fatty acid classes were not influenced by the dietary PSA, and the n-3/n-6 ratio of fish fillets remained at approximately 1 (Table 5).

Table 2. Fatty Acid Composition (Percent of Total Fatty Acids) of Experimental Diets

	experimental diets[a]		
fatty acid	0% PSA	10% PSA	20% PSA
14:0	0.19	0.19	0.21
15:0	–[b]	–	–
16:0	5.69	5.44	5.41
16:1n-7	–	–	–
17:0	–	–	–
16:3n-4	–	–	–
17:1n-7	–	–	–
18:0	2.85	2.70	2.55
18:1n-9	38.25	29.16	20.55
18:1n-7	2.10	1.39	0.83
18:1n-12	–	10.81	20.12
18:2n-6	25.39	25.29	25.50
18:3n-6	0.19	–	–
18:3n-4	–	–	–
18:3n-3	24.35	24.42	24.55
18:4n-3	–	–	–
20:0	0.29	0.19	0.09
20:1n-9	0.44	0.18	–
20:2n-6	–	–	–
21:0	–	–	–
20:4n-6	–	–	–
20:3n-3	–	–	–
20:4n-3	–	–	–
20:5n-3	–	–	–
22:0	0.25	0.24	0.19
22:4n-6	–	–	–
22:5n-3	–	–	–
22:6n-3	–	–	–
24:1n-9	–	–	–
total SFA[c]	9.27	8.75	8.45
total MUFA[d]	40.79	41.54	41.50
total PUFA[e]	49.94	49.71	50.05
total LC-PUFA[f]	–	–	–
18:3n-3/18:2n-6	0.96	0.97	0.96

[a]See Table 1 footnote *a*. [b]Not detected. Some fatty acids were not detected in the experimental diets, but because they were present in fish tissues, they were reported in this table for comparison. [c]SFA, saturated fatty acid. [d]MUFA, monounsaturated fatty acid. [e]PUFA, polyunsaturated fatty acid. [f]LC-PUFA, long-chain polyunsaturated fatty acid.

In the liver lipids, the n-3 PUFA and LC-PUFA contents were significantly higher in fish fed the dietary PSA treatment (Table 6). Livers of fish fed diets containing PSA had a DHA content that was 1.6-fold higher and significantly different from that of fish fed the 0% PSA diet. Likewise, fish fed the PSA-supplemented diets had a significantly higher EPA+DHA content than those fed the 0% PSA diet. The liver PSA content increased as the dietary PSA increased. The liver fatty acid ratio of n-3/n-6 was lower than that of diets. Dietary PSA increased the total PUFA but decreased the total SFA content in fish livers (Table 6).

By comparison of the fatty acid profiles between liver and fillet lipids, SFA and MUFA were higher in the former, whereas PUFA, such as 18:3n-3, 18:2n-6, EPA, DHA, and ARA, were markedly higher in the fillet than in the liver. In both the fillet and liver, the ratio of 18:3n-3/18:2n-6 was lower than that of

Table 3. Growth Performance, Feed Utilization Efficiency, and Biological Indices of Fish[a]

	experimental diets[b]		
	0% PSA	10% PSA	20% PSA
initial weight (g)	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0
final weight (g)	39.0 ± 1.2	39.6 ± 1.9	39.6 ± 3.0
weight gain (%)[c]	787.9 ± 26.2	801.6 ± 43.6	801.7 ± 67.5
SGR[d]	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.1
HSI[e]	1.1 ± 0.1	1.0 ± 0.0	1.2 ± 0.1
VSI[f]	3.8 ± 0.0	3.6 ± 0.0	4.0 ± 0.2
IPF[g]	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
GSI[h] female	0.2 ± 0.2	0.5 ± 0.4	0.3 ± 0.1
GSI male	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
FCR[i]	1.1 ± 0.0	1.2 ± 0.1	1.2 ± 0.0
condition factor[j]	2.1 ± 0.0	2.0 ± 0.0	2.1 ± 0.0
survival rate[k] (%)	97.8 ± 2.2	95.6 ± 4.4	97.8 ± 2.2
hematocrit[l] (%)	30.4 ± 1.5	29.6 ± 1.8	30.4 ± 0.6
$ADC_{dry\ matter}$[m] (%)	65.0 ± 1.6	62.0 ± 1.4	70.7 ± 3.7
ADC_{lipid}[n] (%)	91.1 ± 0.3	91.1 ± 1.3	94.0 ± 1.0

[a]Values are the mean ± SE of quadruplicate groups of fish. [b]See Table 1 footnote *a*. [c]Percentage weight gain = (final weight − initial weight)/initial weight × 100. [d]SGR, specific growth rate = [(ln final mean weight − ln initial mean weight)/days of feeding trial] × 100. [e]HSI, hepatosomatic index = [liver weight (g)/body weight (g)] × 100. [f]VSI, viserosomatic index = [visceral weight (g)/body weight (g)] × 100. [g]IPF, intraperitoneal fat index = [intraperitoneal fat weight (g)/body weight (g)] × 100. [h]GSI, gonadosomatic index = [gonad weight (g)/body weight (g)] × 100. [i]FCR, feed conversion ratio = total dry feed fed (g)/wet weight gain (g). [j]Condition factor = [final body weight/(total length)^3] × 100. [k]Survival = (final fish number/initial fish number) × 100. [l]Hematocrit = [height of packed red cells (mm)/height of packed red cells and plasma (mm)] × 100. [m]Apparent digestibility coefficient of dry matter. [n]Apparent digestibility coefficient of lipid.

Table 4. Whole-Body Composition (Percent Wet Weight Basis) of Tilapia Fed Diets with Increasing Petroselinic Acid[a]

		experimental diets[b]		
component	initial	0% PSA	10% PSA	20% PSA
moisture	80.8	74.7 ± 0.3 a	75.7 ± 0.5 ab	76.1 ± 0.3 b
crude protein	13.6	14.6 ± 0.1	14.2 ± 0.3	14.2 ± 0.4
crude lipid	2.3	7.8 ± 0.3	6.8 ± 0.4	7.0 ± 0.1
ash	3.8	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.0

[a]Values are the mean ± SE of quadruplicate groups of fish. Different letters in the same row indicate significant difference at $P < 0.05$. Initial fish was excluded in statistical analysis. [b]See Table 1 footnote *a*.

the experimental diets with values 0.71−0.73 and 0.45−0.50, respectively. In both the fillet and liver, increasing dietary PSA level resulted in significantly increased PSA and decreased 18:1n-9 contents. Across all treatments, 18:1n-9 was still the most dominant fatty acid in fillet and liver, which ranged from 24.1 to 33.8% and from 34.3 to 40.4% of total fatty acids, respectively. However, the relative content of PSA in the fillet was somewhat higher than that of the liver.

The fatty acid composition of the fish whole-body lipids at the end of the feeding trial is reported as milligrams per 100 g fish in Table 7. The fatty acid classes of fish were affected by dietary PSA. Total SFA was highest (1547.2 mg/100 g fish) and lowest (1234.0 mg/100 g fish) for fish fed the 0% PSA and 20% PSA diets, respectively, which were significantly different from

Table 5. Fillet Fatty Acid Composition (Percent Fatty Acid) and Lipid Content of Tilapia Fed Increasing Dietary Petroselinic Acid[a]

	experimental diets[b]		
fatty acid	0% PSA	10% PSA	20% PSA
14:0	1.33 ± 0.05	1.42 ± 0.07	1.50 ± 0.04
15:0		0.12 ± 0.06	
16:0	16.27 ± 0.05	16.91 ± 0.29	16.99 ± 0.33
16:1n-7	2.53 ± 0.07 a	3.25 ± 0.05 b	3.63 ± 0.30 b
17:0		0.08 ± 0.04	
16:3n-4	0.20 ± 0.02	0.20 ± 0.02	0.09 ± 0.09
17:1n-7	0.44 ± 0.04	0.26 ± 0.13	0.40 ± 0.05
18:0	4.96 ± 0.14	5.08 ± 0.05	4.85 ± 0.05
18:1n-9	33.87 ± 0.89 c	27.70 ± 0.64 b	24.14 ± 0.62 a
18:1n-7	2.88 ± 0.12 b	2.58 ± 0.07 ab	2.39 ± 0.12 a
18:1n-12		5.17 ± 0.06 a	9.77 ± 0.21 b
18:2n-6	13.86 ± 0.19 b	12.72 ± 0.14 a	12.04 ± 0.35 a
18:3n-6	0.90 ± 0.15	0.92 ± 0.02	0.80 ± 0.08
18:3n-4		0.12 ± 0.01	0.21 ± 0.11
18:3n-3	10.12 ± 0.19 b	9.05 ± 0.26 a	8.82 ± 0.25 a
18:4n-3	0.68 ± 0.05 b	0.56 ± 0.02 ab	0.42 ± 0.06 a
20:0	0.12 ± 0.06		
20:1n-9	0.94 ± 0.05 b	0.82 ± 0.06 ab	0.72 ± 0.07 a
20:2n-6	0.37 ± 0.11	0.39 ± 0.05	0.35 ± 0.03
20:3n-6	0.59 ± 0.02 a	0.74 ± 0.03 b	0.72 ± 0.05 b
20:4n-6	1.42 ± 0.06	1.69 ± 0.12	1.75 ± 0.10
20:3n-3	0.98 ± 0.08 b	0.76 ± 0.05 a	0.86 ± 0.02 ab
20:4n-3	0.20 ± 0.03	0.12 ± 0.06	0.22 ± 0.03
20:5n-3	0.31 ± 0.12	0.33 ± 0.04	0.21 ± 0.10
22:4n-6	0.42 ± 0.02	0.48 ± 0.08	0.57 ± 0.04
22:5n-3	0.83 ± 0.04 a	0.99 ± 0.03 ab	1.05 ± 0.07 b
24:0	0.12 ± 0.06		0.11 ± 0.05
22:6n-3	3.69 ± 0.38	4.81 ± 0.43	4.85 ± 0.25
total SFA	22.80 ± 0.07	23.61 ± 0.29	23.45 ± 0.38
total MUFA	40.65 ± 0.89	39.77 ± 0.74	41.05 ± 0.66
total PUFA	34.56 ± 0.86	33.88 ± 0.41	32.95 ± 0.70
total LC-PUFA	8.43 ± 0.51	9.92 ± 0.73	10.22 ± 0.26
total n-3 PUFA	16.80 ± 0.60	16.63 ± 0.27	16.42 ± 0.59
total n-6 PUFA	17.56 ± 0.24 b	16.93 ± 0.13 ab	16.22 ± 0.23 a
total n-3 LC-PUFA	6.00 ± 0.46	7.02 ± 0.51	7.18 ± 0.39
total n-6 LC-PUFA	2.42 ± 0.05 a	2.90 ± 0.23 ab	3.04 ± 0.12 b
n-3/n-6 PUFA	0.96 ± 0.02	0.98 ± 0.01	1.01 ± 0.03
EPA+DHA	3.99 ± 0.47	5.14 ± 0.44	5.06 ± 0.33
lipid content	1.71 ± 0.06	1.66 ± 0.07	1.53 ± 0.05

[a]Values are the mean ± SE of quadruplicate groups of fish. Different letters in the same row indicate significant difference at $P < 0.05$. [b]See Table 1 footnote *a*.

each other. The highest total PUFA content was recorded in fish fed the 0% PSA diet (2062.5 mg/100 g fish) and significantly higher than in those fed the 10% PSA (1649.1 mg/100 g fish) or 20% PSA diet (1555.2 mg/100 g fish). Likewise, total n-3 PUFA and n-6 PUFA of fish showed a similar trend whereby dietary PSA content significantly decreased the content of both n-3 and n-6 PUFA. Among all of the fatty acids, 18:1n-9 was highest in the fish whole-body lipids with values from 1414.4 ± 92.5 to 2481.6 ± 120.5 mg/100 g fish, followed by 16:0 at values from 885.3 ± 23.5 to 1117.6 ± 49.9 mg/100 g fish. Fish fed the PSA diets recorded significantly

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Table 6. Liver Fatty Acid Composition (Percent Fatty Acid) and Lipid Content of Tilapia Fed Increasing Dietary Petroselinic Acid[a]

	experimental diets[b]		
fatty acid	0% PSA	10% PSA	20% PSA
14:0	3.04 ± 0.15	3.08 ± 0.20	2.95 ± 0.29
14:1	0.09 ± 0.01		
16:0	24.18 ± 0.82 b	21.14 ± 0.51 a	22.08 ± 0.82 ab
16:1n-7	4.42 ± 0.16	4.36 ± 0.53	4.83 ± 0.11
17:0	0.10 ± 0.01	0.10 ± 0.00	0.09 ± 0.02
17:1n-7	0.07 ± 0.01		
18:0	9.16 ± 0.54	8.08 ± 0.68	8.47 ± 0.49
18:1n-9	40.39 ± 1.10 c	37.39 ± 0.28 b	34.38 ± 0.14 a
18:1n-7	3.49 ± 0.07 b	3.06 ± 0.10 a	3.34 ± 0.05 b
18:1n-12		3.86 ± 0.14 a	6.84 ± 0.85 b
18:2n-6	4.73 ± 0.29	5.85 ± 0.08	4.68 ± 0.78
18:3n-6	0.44 ± 0.06	0.51 ± 0.05	0.44 ± 0.08
18:3n-4	0.02 ± 0.01 a	0.28 ± 0.01 b	0.38 ± 0.01 c
18:3n-3	2.16 ± 0.24	2.95 ± 0.06	2.25 ± 0.48
18:4n-3	0.14 ± 0.02	0.17 ± 0.04	0.18 ± 0.02
20:0	0.16 ± 0.01 b	0.13 ± 0.03 ab	0.10 ± 0.01 a
20:1n-9	1.36 ± 0.05 b	1.29 ± 0.11 ab	1.04 ± 0.03 a
20:2n-6	0.32 ± 0.04	0.33 ± 0.03	0.28 ± 0.03
20:3n-6	0.33 ± 0.04	0.37 ± 0.04	0.37 ± 0.05
20:4n-6	0.73 ± 0.14	1.04 ± 0.03	1.14 ± 0.14
20:3n-3	0.30 ± 0.05	0.41 ± 0.05	0.26 ± 0.05
20:4n-3	0.07 ± 0.00	0.09 ± 0.02	0.04 ± 0.04
20:5n-3	0.18 ± 0.04	0.26 ± 0.01	0.16 ± 0.06
22:0	0.08 ± 0.02		
22:1n-9	0.03 ± 0.01		
22:4n-6	0.13 ± 0.03	0.16 ± 0.01	0.19 ± 0.05
22:5n-3	0.22 ± 0.04	0.29 ± 0.01	0.30 ± 0.06
22:6n-3	1.48 ± 0.20 a	2.54 ± 0.05 b	2.34 ± 0.26 b
24:1n-9	0.07 ± 0.02		
total SFA	36.71 ± 1.22	32.53 ± 0.82	33.68 ± 1.62
total MUFA	49.91 ± 0.96	49.96 ± 0.66	50.43 ± 0.59
total PUFA	11.25 ± 0.89 a	15.23 ± 0.10 b	13.02 ± 1.35 ab
total LC-PUFA	3.43 ± 0.48 a	5.14 ± 0.12 b	4.81 ± 0.49 ab
total n-3 PUFA	4.54 ± 0.46 a	6.70 ± 0.03 b	5.53 ± 0.60 ab
total n-6 PUFA	6.68 ± 0.44	8.26 ± 0.10	7.11 ± 0.80
total n-3 LC-PUFA	2.24 ± 0.28 a	3.58 ± 0.11 b	3.10 ± 0.28 b
total n-6 LC-PUFA	1.19 ± 0.44	1.57 ± 0.06	1.70 ± 0.21
n-3/n-6 PUFA	0.68 ± 0.02 a	0.81 ± 0.01 b	0.78 ± 0.05 ab
EPA+DHA	1.65 ± 0.23 a	2.79 ± 0.05 b	2.51 ± 0.26 b
lipid content	15.83 ± 0.67	13.39 ± 0.61	13.43 ± 1.08

[a]Values are the mean ± SE of quadruplicate groups of fish. Different letters in the same row indicate significant difference at $P < 0.05$. [b]See Table 1 footnote *a*.

lower 16:0 contents than fish fed the 0% PSA diet. The amount of 20:1n-9 showed significant differences among all of the treatments, with fish fed the 10% PSA having significantly lower and higher 20:1n-9 content compared to fish fed the 0% PSA and 20% PSA diets, respectively. The PSA content in 20% PSA-fed fish was double that of fish fed the 10% PSA diet (Table 7).

Regardless of the dietary treatment, the amount of 18:2n-6 in fish whole-body lipids was about 1.4-fold higher than that of 18:3n-3. Fish fed the 0% PSA diet had significantly higher contents of 18:3n-3, 18:2n-6, 20:4n-3, and 20:3n-6 than those fed the PSA-added diets. For 18:4n-3, 20:3n-3, and 18:3n-6, fish fed the 0% PSA diet had significantly higher content than that of fish fed 20% PSA but not significantly higher than that of fish fed 10% PSA. The whole-body EPA+DHA content was not significantly different for all of the dietary treatments (Table 7).

In Vivo Fatty Acid Metabolism. Table 8 shows the summary of the total apparent in vivo fatty acid neogenesis, β-oxidation, desaturase, and elongase activities in tilapia after 59 days of the feeding trial. Total apparent in vivo β-oxidation was significantly higher in fish fed dietary PSA, whereas fish fed the 0% PSA diet had significantly higher fatty acid neogenesis and elongase activity than PSA-fed fish. Fatty acid elongase activity acting on n-3 PUFA and n-6 PUFA was not significantly affected by dietary PSA supplementation. The overall enzyme activities acting on n-3 PUFA were greater than those on n-6 PUFA across all dietary treatments.

Regardless of dietary treatment, the apparent in vivo elongase activity on 14:0 was the highest among all of the fatty acids with values from 0.618 ± 0.063 to 1.121 ± 0.096 μmol/g/day, followed by 18:4n-3, 20:5n-3, 22:5n-3, 18:3n-6, 16:0, 16:1n-7, 18:3n-3, and 18:1n-9 (Figure 1). For both 14:0 and 16:0, fish fed dietary PSA had significantly lower elongase activity compared to fish fed the 0% PSA. Fish fed the 20% PSA diet showed significantly lower 18:3n-3 elongated to the dead-end product of 20:3n-3 than fish the fed 0% PSA diet. Elongations of other fatty acids were relatively low, and no significant effect of dietary PSA was observed.

The apparent in vivo Δ-9, Δ-6, and Δ-5 desaturase activities are reported in Figure 2. No significant differences were detected for the apparent in vivo desaturase activity, except for the Δ-6 desaturase activity on 18:3n-3, for which fish fed the PSA supplemented diets were observed to express lower enzyme activity compared to fish fed the 0% PSA diet. Despite a gradual decrease in Δ-6 desaturase activity on 18:2n-6 with increasing dietary PSA, the differences were not statistically significant. In all dietary treatments, a 1.4-fold higher Δ-6 desaturase activity was on 18:3n-3 than on 18:2n-6. Meanwhile, there was a 2.8-fold higher Δ-5 desaturase activity acting on 20:4n-3 than on 20:3n-6. Most of the Δ-9 desaturase activity acted on 16:0 with values ranging from 0.254 ± 0.053 to 0.273 ± 0.001 μmol/g/day. No Δ-9 desaturase activity was detected on 18:0.

Across all treatments, 18:3n-3 had the highest apparent in vivo β-oxidation, followed by 18:2n-6, PSA. and 18:1n-9 (Figure 3). Significant differences between the dietary treatments were noted on 18:3n-3, which increased from 1.085 ± 0.074 μmol/g/day in the 0% PSA-fed fish to 1.509 ± 0.034 μmol/g/day in 20% PSA-fed fish. Likewise, β-oxidation on 18:2n-6 was significantly higher in fish fed the PSA-supplemented diets. Fish fed the highest dietary PSA content had significantly higher β-oxidation on PSA, which increased about 1.7-fold from 0.621 ± 0.051 (10% PSA diet) to 1.088 ± 0.030 μmol/g/day (20% PSA diet). 18:0, 20:0, 22:0, 22:1n-9, and 24:1n-9 showed minimal activity of this enzyme, and no β-oxidation was detected for the other fatty acids (data not shown).

The apparent in vivo enzyme activities on n-3 PUFA and n-6 PUFA bioconversion pathway (μmol/g/day) are reported in Figure 4, panels a and b, respectively. Irrespective of the dietary treatment, the Δ-6 desaturase activity on 18:3n-3 was >3-fold higher than on 20:3n-3; 18:2n-6 was Δ-6 desaturated to 18:3n-6 at a 5 times higher rate than being elongated to 20:2n-6. Fish

Table 7. Fatty Acid Content (mg/100 g Fish) of Initial and Final Fish Whole-Body[a]

		experimental diets[b]		
fatty acid	initial	0% PSA	10% PSA	20% PSA
14:0	27.0	115.2 ± 3.4	119.5 ± 14.5	115.3 ± 0.3
14:1n-5		1.7 ± 1.7	1.2 ± 1.2	2.2 ± 1.2
15:0	3.2		2.0 ± 1.2	1.6 ± 1.6
16:0	342.9	1117.6 ± 49.9 b	954.9 ± 95.6 ab	885.3 ± 23.5 a
16:1n-7	59.5	209.4 ± 9.8	198.9 ± 28.7	185.6 ± 1.8
17:0	5.7	6.2 ± 1.0	5.4 ± 1.2	4.6 ± 1.1
16:3n-4		1.7 ± 1.7	1.4 ± 0.7	
17:1n-7		2.9 ± 2.9	1.5 ± 1.5	2.3 ± 2.3
18:0	105.3	282.5 ± 15.6 b	222.7 ± 18.4 a	209.8 ± 11.9 a
18:1n-9	367.3	2481.6 ± 120.5 b	1786.5 ± 161.8 a	1414.4 ± 92.5 a
18:1n-7	53.5	191.8 ± 8.3 b	144.9 ± 14.6 a	122.1 ± 6.8 a
18:1n-12			317.9 ± 12.1 a	625.2 ± 45.6 b
18:2n-6	201.6	933.6 ± 37.4 b	751.5 ± 41.4 a	710.1 ± 50.4 a
18:3n-6	4.8	66.7 ± 10.1 b	48.1 ± 2.6 ab	43.5 ± 1.5 a
18:3n-4			7.9 ± 0.8 a	13.2 ± 1.1 b
18:3n-3	14.5	684.5 ± 33.0 b	532.0 ± 29.8 a	483.4 ± 39.1 a
18:4n-3		45.6 ± 6.6 b	31.7 ± 2.8 ab	26.2 ± 0.1 a
20:0	3.7	13.8 ± 0.6 c	8.4 ± 0.6 b	6.2 ± 0.3 a
20:1n-9	16.8	76.1 ± 2.7 c	54.0 ± 3.5 b	41.7 ± 2.2 a
20:2n-6	11.6	25.8 ± 0.8	20.4 ± 1.9	20.4 ± 1.7
20:3n-6	10.3	28.2 ± 1.2 b	21.7 ± 2.4 a	22.6 ± 0.7 a
21:0			1.6 ± 1.6	0.7 ± 0.7
20:4n-6	31.1	40.9 ± 0.5	34.9 ± 2.9	35.7 ± 1.8
20:3n-3	4.5	52.0 ± 2.4 b	42.2 ± 2.5 ab	40.1 ± 3.8 a
20:4n-3		12.4 ± 0.4 b	8.0 ± 1.1 a	8.3 ± 0.5 a
20:5n-3		11.5 ± 2.2	10.3 ± 0.6	10.1 ± 0.7
22:0	3.7	5.9 ± 1.4	5.3 ± 0.5	5.2 ± 0.4
22:1n-9	2.0	1.7 ± 0.9		
22:2n-6				4.7 ± 4.7
22:4n-6		14.2 ± 0.8	14.4 ± 2.1	10.7 ± 5.3
22:5n-3	11.6	36.5 ± 2.4	31.6 ± 2.6	31.8 ± 1.6
24:0		5.8 ± 0.1	4.0 ± 2.5	5.4 ± 0.6
22:6n-3	71.1	108.9 ± 3.8	93.0 ± 6.9	94.4 ± 5.4
24:1n-9	12.8			
total SFA	491.5	1547.2 ± 62.7 b	1323.8 ± 131.3 ab	1234.0 ± 34.3 a
total MUFA	511.9	2965.1 ± 138.3	2504.9 ± 215.6	2393.4 ± 146.2
total PUFA	361.1	2062.5 ± 91.0 b	1649.1 ± 92.1 a	1555.2 ± 107.5 a
total LC-PUFA	128.5	304.6 ± 7.3	256.1 ± 18.7	253.8 ± 19.1
total n-3 PUFA	101.7	951.4 ± 45.1 b	748.9 ± 41.2 a	694.4 ± 50.6 a
total n-6 PUFA	259.4	1109.4 ± 47.3 b	891.0 ± 50.9 a	847.7 ± 55.8 a
total n-3 LC-PUFA	87.1	221.3 ± 6.0 b	185.2 ± 11.4 a	184.8 ± 11.5 a
total n-6 LC-PUFA	41.4	83.3 ± 1.5	71.0 ± 7.3	68.9 ± 7.6
n-3/n-6 PUFA	0.4	0.9 ± 0.0 b	0.8 ± 0.0 ab	0.8 ± 0.0 a
EPA+DHA	71.1	120.4 ± 5.9	103.3 ± 7.5	104.5 ± 6.0

[a]Values are the mean ± SE of quadruplicate groups of fish. Different letters in the same row indicate significant difference at $P < 0.05$. Initial fish was excluded in statistical analysis. [b]See Table 1 footnote *a*.

fed 0% PSA diet showed the highest Δ-6 desaturase activity of 18:3n-3 (0.2 μmol/g/day), which was significantly higher than those fed dietary PSA. Likewise, Δ-6 desaturase activity on 18:2n-6 showed the same trend, albeit no significant difference was detected. On the dead-end pathway, fish fed the 20% PSA diet had the lowest amounts of 18:3n-3 being elongated to 20:3n-3, which was significantly lower than that of fish fed 0% PSA diet, but not significantly different from those fed 10% PSA. However, no significant differences were noted on the elongation of 18:2n-6 among the three treatments. Along the n-3 PUFA biosynthetic pathway from 18:3n-3 to DHA, the enzyme activities were decreasing progressively. This trend was also noted on the 18:2n-6 biosynthesis pathway.

The fate of 18:3n-3 and 18:2n-6 toward desaturation, elongation, and β-oxidation is expressed as percentage of the net intake in Figure 5. Regardless of dietary treatment, β-oxidation on 18:3n-3 was >50% of its net intake; <20% of 18:3n-3 intake was desaturated or elongated. 18:2n-6 recorded the same trend as 18:3n-3, whereby β-oxidation accounted for the most percentage and ranged from 46.7 ± 2.8 to 60.8 ± 1.7%, followed by desaturation (4.9−6.2%) and elongation (0.8−1.1%). Overall, apparent in vivo desaturation, elongation,

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Table 8. Total Apparent in Vivo Fatty Acid Neogenesis, β-Oxidation, Desaturation, and Elongation (μmol/g/day) in Tilapia Fed Increasing Dietary Petroselinic Acid[a]

	experimental diets[b]		
	0% PSA	10% PSA	20% PSA
SFA and MUFA			
FA neogenesis	1.262 ± 0.090 b	0.755 ± 0.063 a	0.949 ± 0.041 a
total elongation	1.261 ± 0.128 b	0.696 ± 0.057 a	0.925 ± 0.046 a
total oxidation	0.535 ± 0.163 a	1.331 ± 0.317 b	1.337 ± 0.096 b
total Δ-9 desaturase	0.272 ± 0.020	0.256 ± 0.055	0.276 ± 0.003
n-6 PUFA			
total elongation	0.115 ± 0.002	0.097 ± 0.012	0.103 ± 0.003
total oxidation	1.009 ± 0.073 a	1.382 ± 0.138 b	1.387 ± 0.031 b
total Δ-6 desaturase	0.135 ± 0.011	0.117 ± 0.009	0.113 ± 0.006
total Δ-5 desaturase	0.051 ± 0.000	0.045 ± 0.005	0.043 ± 0.005
n-3 PUFA			
total elongation	0.421 ± 0.012	0.353 ± 0.030	0.366 ± 0.012
total oxidation	1.085 ± 0.074 a	1.480 ± 0.121 b	1.509 ± 0.034 b
total Δ-6 desaturase	0.294 ± 0.016 b	0.239 ± 0.018 a	0.243 ± 0.006 a
total Δ-5 desaturase	0.138 ± 0.007	0.117 ± 0.011	0.122 ± 0.004
total			
neogenesis	1.262 ± 0.090 b	0.755 ± 0.063 a	0.949 ± 0.041 a
oxidation	2.629 ± 0.308 a	4.193 ± 0.556 b	4.232 ± 0.161 b
elongation	1.797 ± 0.139 b	1.145 ± 0.068 a	1.394 ± 0.041 a
Δ-9 desaturase	0.272 ± 0.020	0.256 ± 0.055	0.276 ± 0.003
Δ-6 desaturase	0.429 ± 0.028	0.356 ± 0.027	0.356 ± 0.012
Δ-5 desaturase	0.188 ± 0.007	0.162 ± 0.016	0.166 ± 0.009

[a]Values are the mean ± SE of quadruplicate groups of fish. Different letters in the same row indicate significant difference at $P < 0.05$. [b]See Table 1 footnote *a*.

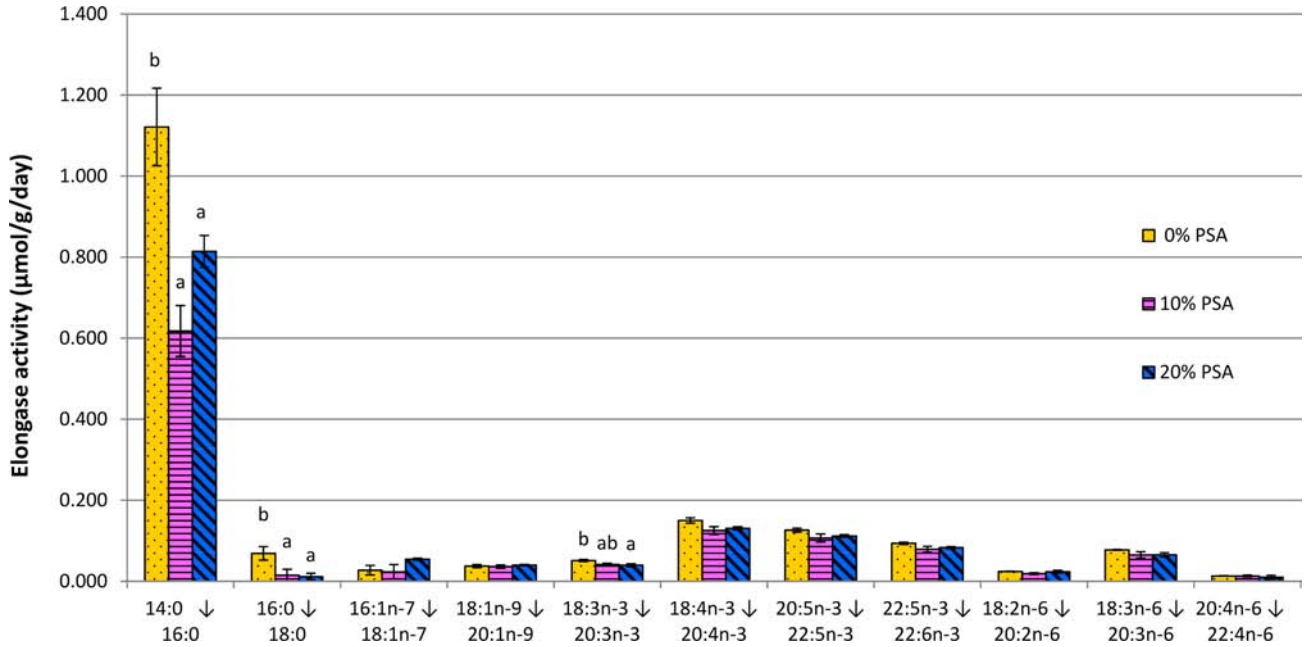

Figure 1. Apparent in vivo elongase activity in tilapia fed increasing dietary petroselinic acid (PSA). Data are expressed as the mean ± SE of quadruplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$). Only major elongase activities were reported.

and β-oxidation on 18:3n-3 were all greater than that of 18:2n-6.

DISCUSSION

The present study examined the effects of dietary PSA on the growth performance, tissue fatty acid composition, and metabolic activities on Nile tilapia. Results showed that all fish had equivalent wet weight growth irrespective of dietary PSA supplementation level. Survival, feed utilization, and biometric parameters were not affected by the dietary treatment, indicating that dietary PSA had no detrimental effect on tilapia (Table 3). The effect of FO replacement on fish health is dependent on the dietary fatty acid composition and

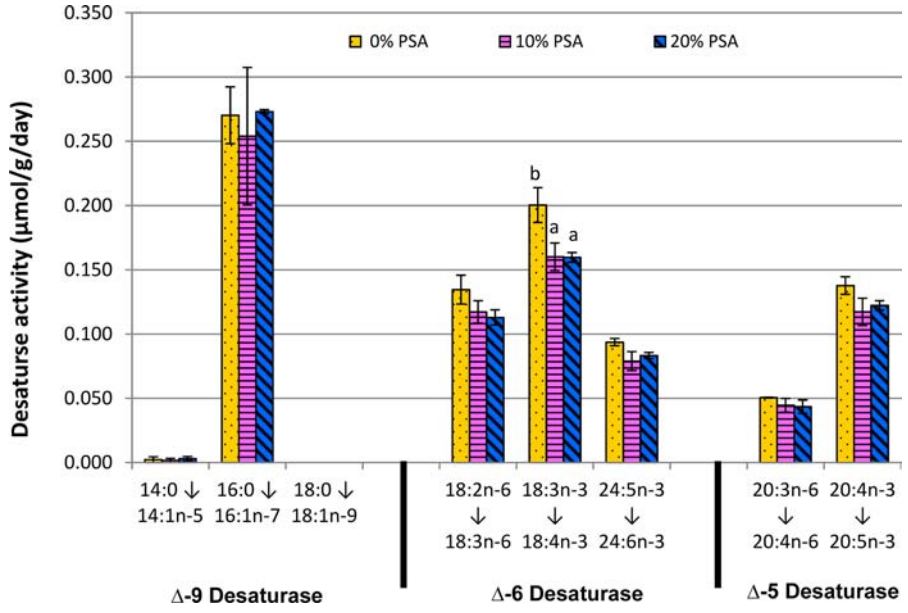

Figure 2. Apparent in vivo desaturase activity in tilapia fed increasing dietary petroselinic acid (PSA). Data are expressed as the mean ± SE of quadruplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$).

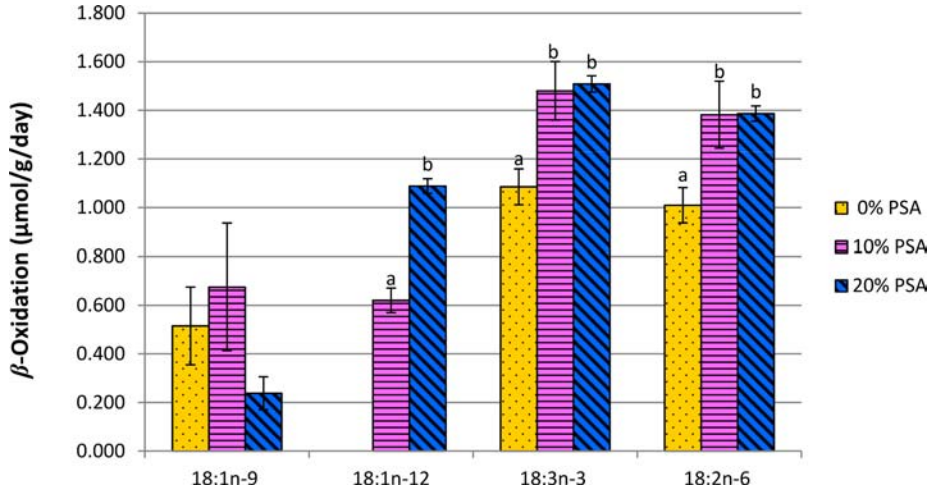

Figure 3. Apparent in vivo fatty acid β-oxidation in tilapia fed increasing dietary petroselinic acid (PSA). Data are expressed as the mean ± SE of quadruplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$). Only fatty acids that were oxidized more than 200 nmol/g/day in at least one diet are reported.

the C_{18} PUFA bioconversion ability of fish,[32] and as long as the essential fatty acid requirements are met, growth performance is generally not negatively affected.

Published information on the digestibility of PSA is contradictory. PSA has been previously demonstrated in vitro to oppose lipolysis because the hydrolysis rate of triacylglycerols (TAG) containing PSA by pancreatic lipase was lower than that of other TAG.[33] In contrast, the absorption of dietary PSA in rats was reported to be as good as that of 18:1n-9.[20] To the best of our knowledge, no studies have been performed on tilapia, and the results of the current study show that the apparent dry matter and lipid digestibility were unaffected by the dietary PSA (Table 3), indicating that inclusion of PSA in FO-deprived diet can be well accepted in fish.

As previously reported,[6,13] the fatty acid composition of diets are not only mirrored in the fatty acid composition of fish but can also affect their in vivo metabolic activities and accumulation of dietary fatty acids. In the present study, all fish were fed almost the same content of dietary fatty acid, with the exception of PSA and 18:1n-9. Thus, the changes in the fatty acid profile of the fish at different PSA supplementations are due to bioaccumulation and/or in vivo metabolism of fatty acids in the fish.

Fish fed the 20% PSA diet showed the highest content of DPA at 1.05%, which was significantly higher compared to fish fed the 0% PSA diet. The DHA and EPA+DHA contents were numerically higher in fillet lipids of fish fed diets containing PSA than those fed the 0% PSA diet, although no significant differences were detected (Table 5). Codabaccus et al.[19] observed that fatty acyl transferase enzymes have an affinity toward deposition and retention of DHA into fillet TAG and polar lipids. A previous study[34] also reported that n-3 PUFA, particulary EPA and DHA, were incorporated into phospholipids at a higher proportion in fillet. It was demonstrated in the current study that dietary PSA supplementation increased the total PUFA in synchrony with decreasing total SFA content in

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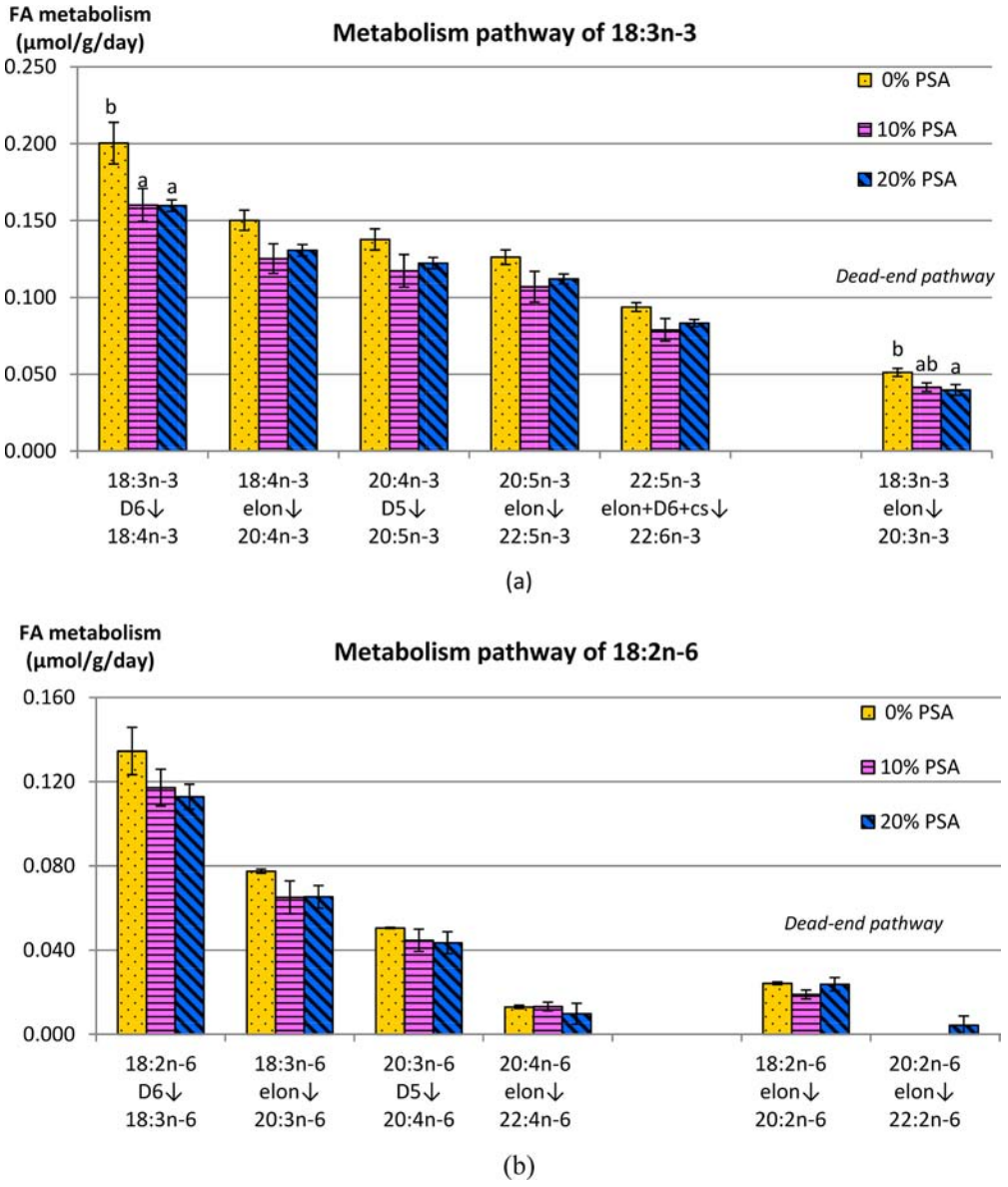

Figure 4. Apparent in vivo elongase and desaturase activity of (a) 18:3n-3 pathway and (b) 18:2n-6 pathway in tilapia fed increasing dietary petroselinic acid (PSA). Data are expressed as the mean ± SE of quadruplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$). D6, Δ-6 desaturase; D5, Δ-5 desaturase; elon, elongase; cs, chain shortening.

the tilapia. In all treatments, the 18:2n-6 content in fish was consistently higher than that of 18:3n-3, which is ascribed to the tendency of 18:2n-6 toward deposition instead of bioconversion and/or utilization.[5,6] Dietary PSA significantly reduced the whole-body 16:0 content and then caused a reduced total SFA content compared to fish fed diets with no PSA supplementation.

Results from the present study showed that fillet lipids contained a large proportion of PUFA, whereas the liver lipids consisted of higher SFA and MUFA (Tables 5 and 6). This difference in the fatty acid composition between fillet and liver is likely attributable to the distinct lipid distribution in different fish tissues. Indeed, neutral lipids represented the greater part of liver fatty acid composition, whereas the fillet lipids consisted of polar lipids, and the majority of the lipids are substituted by PUFA.[35] Morever, the selectivity of medium-chain PUFA and LC-PUFA incorporation was reflected in phospholipids.[36]

The deposition of PSA in fish tissue and whole-body increased proportionally with increasing dietary levels. However, the 18:2n-6 and 18:3n-3 contents in liver were not significantly affected by dietary PSA, whereas a profound depletion of these fatty acids occurred in the fillet and whole-body lipids of fish fed the PSA diets. Meanwhile, dietary PSA increased the EPA+DHA content in fish tissues with a more pronounced increment in the liver lipids (Tables 5−7). Altogether, these results would suggest that PSA can enhance the incorportion of the 18:3n-3 derived PUFA into lipids of tissues, particularly in the liver. Given that the experimental diets contain no PUFA longer than C_{18}, the possibility of LC-PUFA bioaccumulation in fish from dietary intake could be ignored in the present study. The pronouced increased content of total LC-PUFA in the final fish whole-body over initial fish levels with >100 mg EPA+DHA/100 g fish after the feeding period provides conclusive evidence of good LC-PUFA biosynthesis capability in farmed tilapia. This reinforces the

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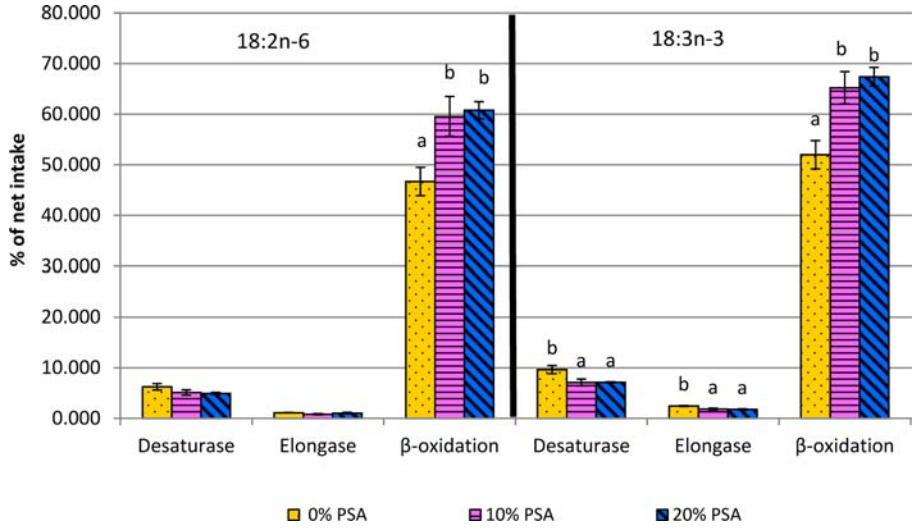

Figure 5. Apparent in vivo desaturation, elongation, and β-oxidation on 18:2n-6 and 18:3n-3 expressed as percent of net intake in tilapia fed increasing dietary petroselinic acid (PSA). Data are expressed as the mean ± SE of quadruplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$).

evidence we have previously reported for the efficiency of farmed tilapia in bioconverting the C_{18} PUFA substrate to the LC-PUFA.[13]

In the present study, fish showed active ex novo biosynthesis; in particular, 16:0 recorded the highest appearance. It has been described[13] that fatty acid neogenesis in fish, to a certain extent, indicates that the total availability of dietary SFA and MUFA is inadequate to meet the requirement of fish for these fatty acids. Nonetheless, fish fed 10% PSA and 20% PSA diets showed significantly lower fatty acid neogenesis activity than fish fed the 0% PSA. In light of the above, it is likely that the presence of PSA somehow mediates the insufficient dietary SFA content in tilapia. Concurrently, dietary PSA significantly augmented the total β-oxidation of SFA and MUFA (Table 8).

In this study, 18:3n-3, 18:2n-6, PSA, and 18:1n-9 were abundant in the diets, and hence these FAs were the major fatty acids undergoing in vivo β-oxidation (Table 2; Figure 3). 18:3n-3, 18:2n-6, and 18:1n-9 were reported to be readily oxidized for energy production when provided in surplus.[8,10,13] Additionally, excessive dietary fatty acid has previously been described to increase the uptake of fatty acids.[37] However, in this study, 18:3n-3 was the most oxidized fatty acid in all dietary treatments, despite the amount of 18:1n-9 being relatively higher in fish fed the control diet. This is in agreement with previous studies in which 18:3n-3 was suggested to be a preferential substrate over 18:2n-6 or 18:1n-9 for β-oxidation.[10,11,17] An in vitro study reported that MUFA, especially 18:1n-9, have a tendency to be oxidized by mitochondria,[38] whereas, notably, the present study observed a comparatively lower β-oxidation of 18:1n-9 in fish fed the PSA diets. In the 10% PSA diet, the quantity of PSA was only one-third of the 18:1n-9, whereas the amounts of these two fatty acids were the same in the 20% PSA diet; however, β-oxidation of PSA was comparable to that of 18:1n-9 in the former and 2-fold higher in the latter, respectively. This clearly indicates that PSA is a favored substrate for β-oxidation, as previously suggested,[21] and surpassed dietary 18:1n-9 for catabolism in tilapia. Consequently, β-oxidation of PSA appeared to be one of the triggers for the significantly higher total β-oxidation in fish fed the PSA diets.

It is known that 18:3n-3 catabolism is proportional to the dietary supply, whereas the β-oxidation activity on 18:2n-6 is independent of substrate availability.[15] However, fish fed dietary PSA recorded increased levels of β-oxidation of 18:3n-3 and 18:2n-6 (Figure 3), resulting in a lower retention of these fatty acids in fish, suggesting that PSA stimulated the total C_{18} PUFA β-oxidation activity. A previous study suggested that the proliferation of both mitochondria and peroxisomes might lead to higher β-oxidation in rat livers;[39] thus, it is provocative to ponder whether PSA acts as a proliferator, binds and activates the peroxisome proliferator-activated receptors-α, and increases the expression of the oxidation enzyme. It has been previously established that increased catabolism of fatty acids would exert a protein sparing effect for muscle growth and thus enhance protein utilization in fish.[38,40] Despite the higher rate of β-oxidation induced by dietary PSA, this did not influence fish growth or feed utilization. However, this should be investigated further and on a longer time scale because tilapia fed the PSA-supplemented diets in the present study tended to have slightly higher growth than those fed the control diet.

Generally, replacing dietary FO with VO or VO blends resulted in higher apparent fatty acid biosynthetic activities in teleosts,[41] including Murray cod,[11,16,17] Atlantic salmon,[7,10,42] rainbow trout,[8] and tilapia.[12,13] In the present study, tilapia exhibited efficient apparent fatty acid bioconversion, whereas the PSA supplementation in the FO-deprived diets significantly reduced the elongation of SFA and MUFA. Among all of the fatty acids, 14:0 was the most elongated fatty acid, which recorded 2-fold higher elongase activity than 16:0 (Figure 1), despite the comparatively higher amount of dietary 16:0 compared to 14:0 in all treatments. However, fish fed the PSA diets had significantly lower apparent in vivo elongase activity on 14:0 and 16:0, denoting that this enzymatic activity on these SFA substrates are directly correlated to the PSA supplementation. Elongase of very long-chain fatty acids (Elovl)-5 is the crucial enzyme in the elongation of C_{18-20} PUFA, whereas Elovl-2 is more capable of elongating C_{20-22} PUFA.[43] In line with a previous study speculating that the efficiency of fish Elovl-5 enzymes is more effective in elongating the n-3 PUFA than the n-6 PUFA,[44] the result of the present study observed

that elongase activities on 18:4n-3 and 18:3n-6 were about 75 and 60% of the desaturase activities on 18:3n-3 and 18:2n-6, respectively (Figure 4), denoting that the affinity of elongase is more toward 18:4n-3 over 18:3n-6.

Stearoyl CoA desaturases (SCDs; namely, Δ-9 desaturase) are the enzymes involved in the synthesis of MUFA; for instance, 18:1n-9 is the product of Δ-9 desaturated 18:0. A study on rainbow trout observed[8] the existence of a greater affinity of Δ-9 desaturase activity toward 18:0 as the Δ-9 desaturated 18:0 was higher than that of 16:0, despite dietary levels of 16:0 being higher than those of 18:0. Conversely, in the present study, the relatively higher dietary 16:0 compared to 18:0 rendered a distinct outcome whereby the highest Δ-9 desaturase activity was recorded in 16:0, whereas no Δ-9 desaturation on 18:0 was observed in any of the dietary treatments (Figure 2). Considering that 18:1n-9 was abundant in all diets, it is conceivable that Δ-9 desaturase activity is dependent upon the availability of enzyme products. Moreover, SCD was not suppressed by 18:1n-9[14] and thus resulted in the majority of Δ-9 desaturation acting on 16:0.

It has been previously reported that tilapia fed a FO-deprived diet showed efficient Δ-5 desaturation of both n-3 and n-6 PUFA.[13] Notably, the present results indicated that the Δ-5 desaturase activity acting on 20:4n-3 was 2-fold greater than that on 20:3n-6 regardless of the dietary treatment (Figure 2). Likewise, a previous study on rainbow trout reported an 8-fold higher Δ-5 desaturase activity on 20:4n-3 than on 20:3n-6. This was attributed to the specificity of Δ-5 desaturase toward 20:4n-3 over 20:3n-6.[45] To maintain the n-3 LC-PUFA content in tissue, the activity of desaturase in fish will be affected by dietary lipid as these are membrane-bound enzymes.[41] Some studies have suggested that the rate of hepatocyte desaturation is influenced by both high C_{18} PUFA substrate supply and low C_{20} and C_{22} LC-PUFA.[42] In addition, the physiological needs of fish for n-3 LC-PUFA might also up-regulate the desaturase gene expression.[46] For fish fed FO-deprived diets, the dietary ratio of 18:3n-3/18:2n-6 has a crucial impact in modulating the Δ-6 desaturase enzymatic activity. In gilthead seabream larvae, an excessively high dietary content of 18:3n-3 inhibited Δ-6 desaturase gene expression.[47] However, Li et al.[48] suggested that a high 18:3n-3/18:2n-6 ratio up-regulated Δ-6 desaturase gene expression in rabbitfish as the fish fed 18:3n-3 rich oil diet (perilla oil) had a higher mRNA level than fish fed dietary high 18:2n-6 (safflower oil). On the other hand, an excessive dietary 18:2n-6 content may inhibit Δ-6 desaturation on 18:3n-3.[49] In the present study, the dietary ratio of 18:3n-3 to 18:2n-6 had been designed as 1 for all treatments, yet the 18:3n-3 showed a higher Δ-6 desaturase activity than that of 18:2n-6, which is in agreement with previous studies on Murray cod[15] and rainbow trout,[45] which showed that Δ-6 desaturase has a higher affinity toward 18:3n-3 because Δ-6 desaturase activity acting on 18:3n-3 is higher than that on 18:2n-6.

Dietary PSA tended to lower the Δ-6 desaturase activity on 18:2n-6, but the ARA content in whole-body fish was only slightly reduced but not significantly (Figure 2; Table 7). However, total n-6 PUFA levels in fillet lipids decreased with increasing levels of dietary PSA. This is somewhat in line with a previous study in which it was reported that rats fed PSA-supplemented diets had significantly lower ARA and n-6 LC-PUFA in the phosphatidylethanolamines and phosphatidylcholines of the brain as PSA, a pseduoproduct mimicking a reduced structure of 18:3n-6, inhibited Δ-6 desaturation of 18:2n-6.[22] However, the reduced Δ-6 desaturase activity on 18:2n-6 in tilapia fed the PSA-added diets did not appear to improve but rather significantly decreased Δ-6 desaturation of 18:3n-3 (Figure 2). A study on Atlantic salmon reported dietary echium oil did not up-regulate the Δ-6 desaturase gene as the relatively high proportion of Δ-6 desaturated fatty acids from diet bypassed the Δ-6 desaturase enzyme through product feedback inhibition.[46] On the basis of the results of the present study, we therefore put forth a hypothesis that feedback inhibition by PSA lowered the Δ-6 desaturase activity on both 18:3n-3 and 18:2n-6 in fish fed dietary PSA, at levels being tested in this study. Taking into consideration that this reduction is antagonistic to the enhancement on β-oxidation, further studies are required to elucidate if the inhibition feedback of PSA increases the β-oxidation of 18:3n-3 and 18:2n-6 or if the PSA induced β-oxidation, but which consequently lowers the availability of the substrate for Δ-6 desaturase enzyme.

Δ-6 Desaturase has been known as a rate-limiting enzyme in the PUFA biosynthetic pathways, and the Δ-6 desaturase activity on 18:3n-3 reached a maximal efficiency at an average substrate level; thus, an excessive dietary 18:3n-3 may have a counterproductive effect in maximizing in vivo n-3 LC-PUFA production.[15,16] Considering that the dietary 18:3n-3 content was the same in all treatments and increasing the dietary PSA level up to 20% did not exacerbate the reduction on desaturation of 18:3n-3, it is likely that Δ-6 desaturase efficiency is a result of a combination of both positive and negative feedback mechanisms. The depletion of the Δ-6 desaturase activity did not profoundly attenuate the subsequent steps of elongation and desaturation (Figure 4a), and the biosythesized LC-PUFA contents in final whole-body were not significantly different across all dietary treatments (Table 7). In light of the above, it is evident that LC-PUFA biosynthesis is not only dictated by Δ-6 desaturase efficiency but also dependent upon Δ-5 desaturase and elongase.

Between the pathways of oxidation and bioconversion of 18:3n-3, β-oxidation accounted for the highest percentage, with <20% of 18:3n-3 net intake being desaturated or elongated (Figure 5). This reinforces the evidence that oxidation for energy production is the paramount fate of 18:3n-3 as reported in previous studies.[7,13] Nevertheless, elongation on 18:3n-3 was significantly lower in fish fed PSA diets compared to the fish fed 0% PSA diet when the elongation rate was expressed as a percentage of net intake. The reduction had been ascribed to the greater β-oxidation of 18:3n-3 caused by dietary PSA. Between the Δ-6 desaturase and elongase pathway of 18:3n-3 and 18:2n-6, these fatty acids were being Δ-6 desaturated at higher rates than being elongated, and this result is in agreement with previous studies[11,17] on Murray cod that reported Δ-6 desaturase as more active than the elongase enzyme.

For all of the enzyme activities, n-3 PUFA was a preferred substrate as the enzyme activity acting on n-3 PUFA was always higher than that of n-6 PUFA. This is consistent with a previous study that reported elongase and desaturase having a greater affinity toward n-3 fatty acids when C_{18} PUFA was supplied in abundance.[11,45] Overall, enzyme activities reduced gradually along the LC-PUFA biosynthetic pathway from 18:2n-6 and 18:3n-3 to ARA and DHA, respectively (Figure 4). This "funnel-like" efficiency was also exhibited in rainbow trout;[8,45] as the chain length increased, the efficiency for bioconversion and associated activities along this pathway decreased.

To the best of our knowledge, this is the first study investigating the effects of dietary PSA supplementation on the

efficiency of fatty acid metabolism in GIFT tilapia. Dietary PSA supplementation did not lead to a detrimental effect on fish growth performance and feed utilization efficiency. Also, the findings gave new insights into the fatty acid metabolism of improved strains of farmed tilapia. Δ-9 Desaturase activity in tilapia was largely determined by the high availability of 18:1n-9. Dietary PSA down-regulated the Δ-6 desaturation of C_{18} PUFA substrates (both 18:3n-3 and 18:2n-6) but increased total β-oxidation in fish. At relatively high dietary levels of PSA tested in the present study, we provided evidence that PSA, a pseudoproduct mimicking the structure of 18:3n-6, did reduce Δ-6 desaturation on 18:2n-6 but, contrary to popular speculation, did not stimulate more Δ-6 desaturase activity on 18:3n-3. We therefore hypothesized that high dietary levels of PSA may potentially inhibit the overall Δ-6 desaturase enzyme activity. Nevertheless, the synthesis of EPA and DHA was not inhibited by the dietary PSA as the biosynthesis of these physiologically important final metabolites is not solely dependent upon the Δ-6 desaturase activity but is also dependent on other fatty acid enzyme activities. In fillet and liver lipids, the deposition of EPA+DHA was enhanced by selective retention with dietary PSA supplementation in tilapia diets.

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ABBREVIATIONS USED

FO, fish oil; VO, vegetable oils; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; GIFT, genetically improved farmed Nile tilapia; PSA, petroselinic acid; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; DPA, docosapentaenoic acid; TAG, triacylglycerols; Elovl, elongase of very long-chain fatty acids; SCD, stearoyl CoA desaturases

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