JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Impact of Reduced Dietary Levels of Eicosapentaenoic Acid and Docosahexaenoic Acid on the Composition of Skin Membrane Lipids in Atlantic Salmon (*Salmo salar* L.)

Ken Cheng,*[,†] Marta Bou,[‡] Bente Ruyter,[‡] Jana Pickova,[†] Emad Ehtesham,[†] Liang Du,[‡] Claudia Venegas,[§] and Ali A. Moazzami[†]

[†]Department of Molecular Sciences, Uppsala BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7015, 75007 Uppsala, Sweden

[‡]Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima), Box, 210, NO-1431 Ås, Norway

[§]AVS Chile, 5550710 Puerto Varas, Chile

Supporting Information

ABSTRACT: Membrane lipids, including sphingolipids and glycerol-phospholipids, are essential in maintaining the skin's barrier function in mammals, but their composition in fish skin and their response to diets have not been evaluated. This study investigated the impacts of reducing dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on membrane lipids in the skin of Atlantic salmon through a 26 week feeding regime supplying different levels (0−2.0% of dry mass) of EPA/DHA. Ceramide, glucosylceramide, sphingomyelin, sphingosine, and sphinganine in salmon skin were analyzed for the first time. Higher concentrations of glucosylceramide and sphingomyelin and higher ratios of glucosylceramide/ceramide and sphingomyelin/ceramide were detected in the deficient group, indicating interruptions in sphingolipidomics. Changes in the glycerol-phospholipid profile in fish skin caused by reducing dietary EPA and DHA were observed. There were no dietary impacts on epidermal thickness and mucus-cell density, but the changes in the phospholipid profile suggest that low dietary EPA and DHA may interrupt the barrier function of fish skin.

KEYWORDS: *ceramide, DHA, EPA, glycerol-phospholipids, fish-skin health, sphingolipidomics*

1. INTRODUCTION

Certain n-3 long-chain polyunsaturated fatty acids (LC-PUFA), mainly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), have been identified as essential fatty acids (EFA) in the diet of Atlantic salmon (*Salmo salar* L.) for good growth performance, health, and final-product quality.[1−3] Fish oil rich in n-3 LC-PUFA has been used in salmon diets, but as a result of relatively stable fish oil production and growing global demand for farmed fish, the aquaculture industry is facing a challenge in meeting the demand for fish oil in Atlantic salmon production.[4,5] Alternative sources of n-3 LC-PUFA, such as algae, krill, and genetically modified plant oils, have been the subject of extensive research, but so far this has not yielded an economically and ecologically sustainable solution for salmon farming.[6−9] As a result, a reduction in n-3 LC-PUFA levels in salmon feed is currently inevitable. It is important to know the possible impacts of reduced dietary EPA and DHA on salmon growth and health. There is still a knowledge gap in terms of Atlantic salmon requirements for dietary EPA and DHA under different environmental conditions. In controlled environments in tanks on land, 10 g/kg EPA and DHA (1% of feed dry mass) is in general considered to be sufficient.[1,10−12] However, a recent study[11] showed that salmon require above 10 g/kg to maintain fish robustness and good health under demanding environmental conditions in sea cages.

The importance of EPA and DHA on fish performance has been studied previously, mostly focusing on the impacts on fish growth, survival, and early development and on fatty acid (FA) composition in fish liver and muscle.[1,2,13−15] Very few experiments have investigated the effects of dietary EPA and DHA on fish-skin health.[11] As with terrestrial-vertebrate skin, fish skin acts as the main barrier to the external environment, maintaining homeostasis in the organism and protecting against potential physical damage and environmental pathogens.[16] However, unlike human skin, the fish epidermis lacks a keratinized layer (stratum corneum) and hairs, and it contains a mucus layer and bone-tissue-related scales.[17] The mucus layer contains antimicrobial and anti-infection enzymes, such as lysozyme, protease, and immunoglobulin, which are important for fish-skin health.[17−19] Their immunological enzyme activities have been found to be implicated in fish epidermis histological parameters, such as epidermal thickness and mucus-cell density.[20,21] During a 6 week experimental-infection period, the density of mucus cells, mainly goblet cells, was found to be positively correlated with epidermal-layer thickness and negatively correlated with parasite density.[20,22]

Received: June 1, 2018
Revised: July 16, 2018
Accepted: July 25, 2018
Published: July 25, 2018

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

The permeability barrier of skin is primarily localized at the stratum corneum in terrestrial vertebrates. Ceramide (Cer), composed of a sphingosine (So) and a fatty acid (FA), is the main lipid (>50% of total lipid mass) in the stratum corneum.[23] It has been reported that EFA deficiency results in impaired sphingolipid metabolism, such as in the conversion of sphingomyelin (Sph) and glucosyl-ceramide (GlcCer) into Cer, leading to abnormal permeability-barrier function in mammal epidermises.[24,25] Although fish skin is unlike the skin of terrestrial vertebrates in structure, many essential functions are shared, such as the mechanical- and chemical-barrier formations that maintain osmotic homeostasis.[17] To the best of our knowledge, only one previous publication has determined the total content of sphingolipids, including Sph and GlcCer, in fish skin: a study on Pacific saury (*Cololabis saira*) using high-performance liquid chromatography (HPLC) coupled with evaporative-light-scattering detection (ELSD).[26] The composition and function of Cer and related sphingolipid metabolites, such as Sph, GlcCer, So, and sphinganine (Sa), in fish skin and their responses to dietary treatments are still unknown.

Glycerol-phospholipids (GPL), including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI), are other important types of membrane lipids in the epidermis.[27] The PUFA in GPL are essential components for maintaining the fluidity of cell membranes, which is important for signal transduction and substance transportation.[28,29] Lowered levels of n-6 PUFA, especially arachidonic acid (20:4n-6), and elevated levels of monounsaturated fatty acids (MUFA) have been observed in epidermal PC and PE in patients with atopic dermatitis.[30] Moreover, a study on rainbow trout (*Oncorhynchus mykiss*) showed that an EFA-deficient diet containing 93.4% saturated FA strongly influenced the GPL composition in fish skin, although no changes were detected in the permeability to water.[29] The function and biosynthesis of GPL in fish skin is still not clear, which makes it interesting to determine the FA composition in GPL subclasses in skin when fish are fed diets deficient in EPA and DHA.

The aim of the present study was thus to investigate the impacts of lowering dietary EPA and DHA levels on the phospholipids in the skin of Atlantic salmon. The composition of sphingolipids and FA composition in GPL subclasses (PC, PE, PS, and PI) in skin and epidermal histological parameters (epidermal thickness and goblet-cell density) were examined. The effects of feeding duration were also evaluated.

2. MATERIALS AND METHODS

2.1. Fish-Feed Formulation. Thirteen experimental diets with different levels of EPA and DHA were formulated in the study. The feed ingredients are thoroughly described in another paper.[12] Briefly, the experimental diets were isoproteic (46.6−47.0%), isolipidic (24.6−25.9%), and isoenergetic (22.1−22.6 MJ/kg) but contained 0, 0.5, 1.0, 1.5, or 2.0% (of feed dry weight) only EPA, only DHA, or a 1:1 mixture of EPA and DHA (EPA+DHA, Table 1). Among these, a diet completely depleted in EPA and DHA (0% EPA+DHA) was used as a negative-control diet. The experimental diets were fishmeal- and fish-oil-free but carefully designed to meet fish-nutritional requirements. Blended poultry oil and rapeseed oil (1:1), which are naturally lacking in EPA and DHA, were used as basic lipid sources in the experimental feeds. EPA and DHA oil concentrates in the form of triacylglycerol (Croda Chemicals Europe Ltd., East Yorkshire, U.K.) were used to control dietary levels of EPA and DHA. All experimental diets were produced by the Nofima feed technology center (Bergen, Norway).

Table 1. Experimental Diets

experimental diet	number of tanks
0% EPA+DHA	3
0.5% EPA	2
1.0% EPA	2
1.5% EPA	2
2.0% EPA	3
0.5% DHA	2
1.0% DHA	2
1.5% DHA	2
2.0% DHA	3
0.5% EPA+DHA	2
1.0% EPA+DHA	2
1.5% EPA+DHA	2
2.0% EPA+DHA	3
CC[a]	3

[a]Commercial-type control diet.

The measured chemical composition and gross energy in the experimental fish feeds are provided in Table S1.

A diet resembling a commercial diet with a 2.2% 1:1 mixture of EPA and DHA (BioMar, Trondheim, Norway) was included as a commercial-type control (CC), in which 26% fishmeal and 9.8% fish oil were used. The main purpose of using the CC was to set a benchmark for growth.

The FA compositions in all diets were described in Bou et al.[12] Importantly, the contents of 18:3n-3, the precursor of EPA and DHA in the biosynthetic pathway, was kept at the same level (about 4.7% of total FA) in all diets. The EPA and/or DHA dietary groups contained increasing contents of EPA and DHA, as it was designed, and the 0% EPA+DHA diet had little EPA (0.05% of total FA) and DHA (0.08% of total FA).

2.2. Experimental Design. The feeding-trial conditions are described in detail in Bou et al.[12] In brief, Atlantic salmon with a mean initial body weight of 52.8 g were randomly distributed into 33 tanks with 70 fish per tank (2 tanks per diet for the 0.5, 1.0, and 1.5% dietary groups and 3 tanks per diet for the CC, 0% EPA+DHA, and 2.0% dietary groups; Table 1) and reared at Nofima Institute in Sunndalsøra, Norway, for 26 weeks. All tanks (1 m^2 surface area, 0.6 cm water depth) were supplied with 15 L/min seawater (33 g/L salinity) at ambient temperature. The water temperature varied between 6.3 and 13.8 °C and the oxygen-saturation level was kept above 85%. Prior to the experiment, the fish were fed a commercial diet (Skretting, Stavanger, Norway) and treated with light to induce smoltification. The feed ration was 15−20% higher than the assessed feed intake and was supplied by automatic belt feeders.

Skin samples for lipid analysis were collected twice, following the same sampling procedures, when fish reached a body weight of 182.9 ± 69.3 g (referred to as 200 g) after 19 weeks of feeding and when they reached a body weight of 379.7 ± 96.5 g (referred to as 400 g) after 26 weeks of feeding. Five fish were randomly selected from each tank and killed using overdoses of MS 222 (0.05−0.08 g/L). Skin samples with mucus and scales from the right fillet were dissected from the dorsal fin to the caudal fin, pooled by tank, and homogenized in dry ice. The homogenate was kept at −40 °C, with the bags left open until the dry ice evaporated, and thereafter stored at −80 °C until analysis. The skin covering the white muscle from the Norwegian Quality Cut of the left fillet of the fish was used for histology analysis. Samples were randomly taken at the termination of the experiment (at 400 g after feeding for 26 weeks, n = 5 fish per tank), cut into sizes (approximately 0.5 cm^2) suitable for histological analysis, and fixed in 10% buffered formalin. The experimental procedure was in accordance with the National Guidelines for Animal Care and Welfare published by the Norwegian Ministry of Education and Research.

2.3. Sphingolipidomics Analysis Using LC-QTOF MS. *Sample Preparation.* Fish-skin samples from eight groups (CC, 0% EPA +DHA, 0.5% EPA, 0.5% DHA, 0.5% EPA+DHA, 2.0% EPA, 2.0%

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

DHA, and 2.0% EPA+DHA) at stages 200 and 400 g were subjected to sphingolipidomics analysis using methods described elsewhere.[31,32] In brief, the homogenized, pooled skin samples from five fish per tank were analyzed three times. The homogenate containing an internal-standard cocktail (0.15 nmol of C17 sphingosine, C17 sphinganine, C17 sphingosine-1-phospate, C17 sphinganine-1-phosphate, C12 sphingomyelin, C12 ceramide, C12 glucosyl(β)-ceramide, C12 lactosyl(β)-ceramide, and C12 ceramide-1-phosphate; sphingolipid mix II, LM-6005, Avanti Polar Lipids, Alabaster, AL) was extracted twice, using 3 mL of chloroform/methanol (1:2, v/v) each time, under sonication in a water bath for 30 min at room temperature. The extract was centrifuged (1800*g*, 20 min) at room temperature, and the supernatant was collected.

Because the amount of Sph in the skin samples was much higher than those of the other sphingolipids measured (Cer, So, Sa, and GlcCer), the content of Sph was determined separately. Skin extract (0.25 mL × 2) was transferred to two tubes, one with a C12:0 Sph internal standard (0.17 nmol; Avanti Polar Lipids, Alabaster, AL) and one without. Sample solvent was evaporated under nitrogen, and the sample was redissolved in 0.5 mL ethanol. The remaining skin extract (5.2 mL) was used for quantification of the other sphingolipids. After evaporation, samples were redissolved in 1 mL ethanol. All samples were centrifuged at 12 000*g* for 20 min at 4 °C before analysis.

Liquid-Chromatography–Mass-Spectrometry Analysis. Liquid chromatography–mass spectrometry (LC-MS) was carried out on an HP1100 LC system (Hewlett-Packard, Palo Alto, CA) coupled to an electrospray-ionization–quadropole time-of-flight mass spectrometer (ESI-QTOF MS; Bruker maXis Impact; Bruker Daltonik GmbH, Bremen, Germany). System integrity was controlled by Hystar software (Bruker Daltonik GmbH). A sodium formate solution (4 μL of formic acid, 20 μL of 1 M NaOH, 100 mL of H_2O, and 100 mL of 2-propanol) was used as the MS calibrant to correct for any mass drift in the analyte. The spectra were acquired in positive-ionization mode scanning within an m/z 50–1500 range.

Analyte separation was performed on a hydrophilic-interaction chromatograph (Atlantis silica HILIC column, particle size 3 μm, 2.1 × 150 mm, Waters, Wexford, Ireland). The injection volume was 10 μL, and the column temperature was maintained electronically at 30 °C. The mobile phase consisted of eluent A, 1% (v/v) formic acid and 10 mM ammonium formate in MS-grade water, and eluent B, 0.1% (v/v) formic acid in acetonitrile, at a constant flow rate of 0.25 mL/min. The programmed eluent gradient was initially reduced from 95 to 5% A over 0.5 min, ramped to 60% A over 10 min, held there for 4.5 min, ramped to 5% A over 2 min, and held there for 15 min before the next run. A plasma reference and a sphingolipid-standard mixture (sphingolipid mix II, LM-6005, Avanti Polar Lipids, Alabaster, AL) were run three times throughout the analysis as a quality control to check the stability of the instruments. The MS raw data were calibrated automatically and converted to mzXML files using Compass DataAnalysis software (Bruker Daltonik GmbH). Peak heights gave good linearity when we compared them with the QTOF responses to a standard Cer C17:0 (Larodan AB, Solna, Sweden) at different concentrations (0.1–1 μg/mL). Therefore, the peak heights for the compounds of interest were calculated by Mzmine software (version 2.15) on the basis of their assigned m/z values and retention times. The concentrations of sphingolipids were determined against known amounts of internal standards and expressed in nanomoles per gram of tissue. The contribution from overlapping signals from the ^{13}C isotopes of other compounds was accounted for when relevant.

2.4. Fatty Acid Analysis of Glycerol-Phospholipids Using TLC and GC-FID. *Sample Preparation.* Total lipids in fish-skin samples (2 g, from five fish per tank) were extracted with 50 mL of chloroform/methanol (2:1, v/v) containing 0.07% (w/v) butylated hydroxytoluene as an antioxidant and 6 mL of NaCl (0.9%), according to the method described by Folch et al.[33] The organic phase was collected and dried under a stream of nitrogen. The GPL fraction was separated from the other lipid classes, such as triacylglycerol, diacylglycerol, and free FA, by thin-layer chromatography (TLC; silica-gel 20 × 20 cm plates, Merck, Darmstadt, Germany) using a mixture of petroleum ether, diethyl ether, and acetic acid (113:20:1, v/v/v) as the mobile phase and employing the method described by Bou et al.[12] and Thomassen et al.[13] After drying, the plates were sprayed with 2% 2,7-dichlorofluorecin in 96% ethanol. Lipid classes were identified under ultraviolet (UV) light at 366 nm. The GPL bands were scraped off the plates and soaked in a mixture of chloroform, methanol, acetic acid, and water (50:39:1:10, v/v/v/v) for 4 h at −40 °C to elute the GPL from the silica gel. The GPL fractions were collected after the addition of 0.5 mL of NaCl (0.9%), centrifuged twice at 700*g* for 10 min, and dried under a stream of nitrogen.[12]

The different types of GPL (PC, PE, PS, and PI) were isolated by the second TLC procedure using chloroform/methanol/acetic acid/water (100:75:6:2, v/v/v/v).[12,34] The GPL classes were revealed by spraying with 2% 2,7-dichlorofluorecin in 96% ethanol and detected under UV light at 366 nm by comparing them with an external standard (Nu-chek Prep, Elysian, MN). The GPL bands were then separately scraped off the TLC plates and trans-methylated to FA methyl esters (FAME) with benzene, methanolic HCl, and 2,2-dimethoxypropane (10:10:1, v/v/v) overnight at room temperature.[35] Samples were neutralized with 6% $NaHCO_3$ after methylation. Tricosylic acid (C23:0; Nu-chek Prep, Elysian, MN) was used as an internal standard.

Gas-Chromatography–Flame-Ionization Analysis. The FAME were analyzed using a gas chromatograph (Hewlett-Packard 6890, Palo Alto, CA) equipped with an autoinjector in split mode (HP 7683, Agilent, Avondale, PA), a BPX70 capillary column (SGE Victoria, Australia, 60 m length, 0.25 mm i.d., 0.25 μm thickness), and a flame-ionization detector (Hewlett-Packard 6890).[13] Helium was the carrier gas with a constant flow of 20 mL/min. Both the injector and the detector temperatures were set at 270 °C. The oven temperature was initially held at 50 °C for 1.2 min, then ramped at 4 °C/min to 170 °C, ramped at 0.5 °C/min to 200 °C, and then ramped to the final temperature of 240 °C at a rate of 10 °C/min. The individual FA were identified by comparing the retention times with those of the external standards (Nu-chek Prep, Elysian, MN). Peak areas were integrated using HP ChemStation to calculate the relative FA contents.

2.5. Skin Histological Analysis. Histopathological evaluation was performed on the skin of the fish from the eight treatments (CC, 0% EPA+DHA, 1.0% EPA, 1.0% DHA, 1.0% EPA+DHA, 2.0% EPA, 2.0% DHA, and 2.0% EPA+DHA; n = 10 per dietary group). Paraplast-embedded skin samples were microtome-cut (5 μm) and stained with standard hematoxylin and eosin (Merck KGaA, Darmstadt, Germany). Stained slides were examined using a standard light microscope (Nikon Optiphot, Tokyo, Japan). Images were captured by means of a Micropublisher camera and QCapture software using a 40× objective. Samples were first subjected to a blinded histopathology evaluation, which means that the identities of the samples were hidden; this was followed by a second evaluation after the decoding of the samples, which provided a description per dietary group, to ensure the observations were unbiased. Epidermal thickness and goblet-cell numbers per 100 μm were evaluated using ImageJ (NIH, Bethesda, MD).

2.6. Data Analysis. The Statistical Analysis System (SAS 9.3, SAS Institute, Cary, NC) was used for univariate data analysis within the experimental groups. The FA data in percentages were square-root–arcsine transformed before the test. The data's normality (Anderson–Darling test) and homoscedasticity (Bartlett's test or Levene's test) were checked. If the tests were failed, the initial data were log-transformed and retested. The general linear model was used for statistical comparisons. For comparison of sphingolipid concentrations, two-way ANOVA was used with the diets and sampling times as fixed factors. For comparison of FA compositions in the GPL fractions, data from different sampling times (at 200 and 400 g after feeding for 19 and 26 weeks, respectively) were analyzed separately using one-way ANOVA. For evaluation of the histological parameters, one-way ANOVA was conducted. If the data did not satisfy the test of normality or the test of homoscedasticity, the Mann–Whitney test was applied as a nonparametric test. Furthermore, Tukey's test was employed as a post hoc test against a predefined significance level ($P < 0.05$).

SIMCA-P 13.0 (Umetrics, Umeå, Sweden) was used for multivariate data analysis of the dietary effects on FA composition. All variables were Pareto-scaled. Principal-component-analysis (PCA) models were created to get an overview of the data set and to search for outliers

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

Table 2. Epidermal Thicknesses (μm) and Goblet-Cell Numbers per 100 μm in the Skin of Fish Fed the Commercial-Type Control (CC) and 0, 1.0, and 2.0% EPA and DHA Diets for 26 Weeks[a]

	CC	0% EPA+DHA	1.0% EPA	1.0% DHA	1.0% EPA+DHA	2.0% EPA	2.0% DHA	2.0% EPA+DHA	*P*
epidermal thickness	30.2 ± 4.01	31.5 ± 3.36	26.6 ± 1.81	30.7 ± 1.55	26.2 ± 1.71	40.0 ± 9.93	31.5 ± 1.84	31.6 ± 1.77	0.20
goblet-cell number per 100 μm	2.57 ± 0.31	2.82 ± 0.34	3.79 ± 0.28	4.11 ± 0.28	3.94 ± 0.37	4.17 ± 0.94	3.56 ± 0.35	3.64 ± 0.27	0.27

[a]Means ± SE; n = 10. P values calculated by one-way ANOVA tests (Tukey's test) within all dietary groups except CC.

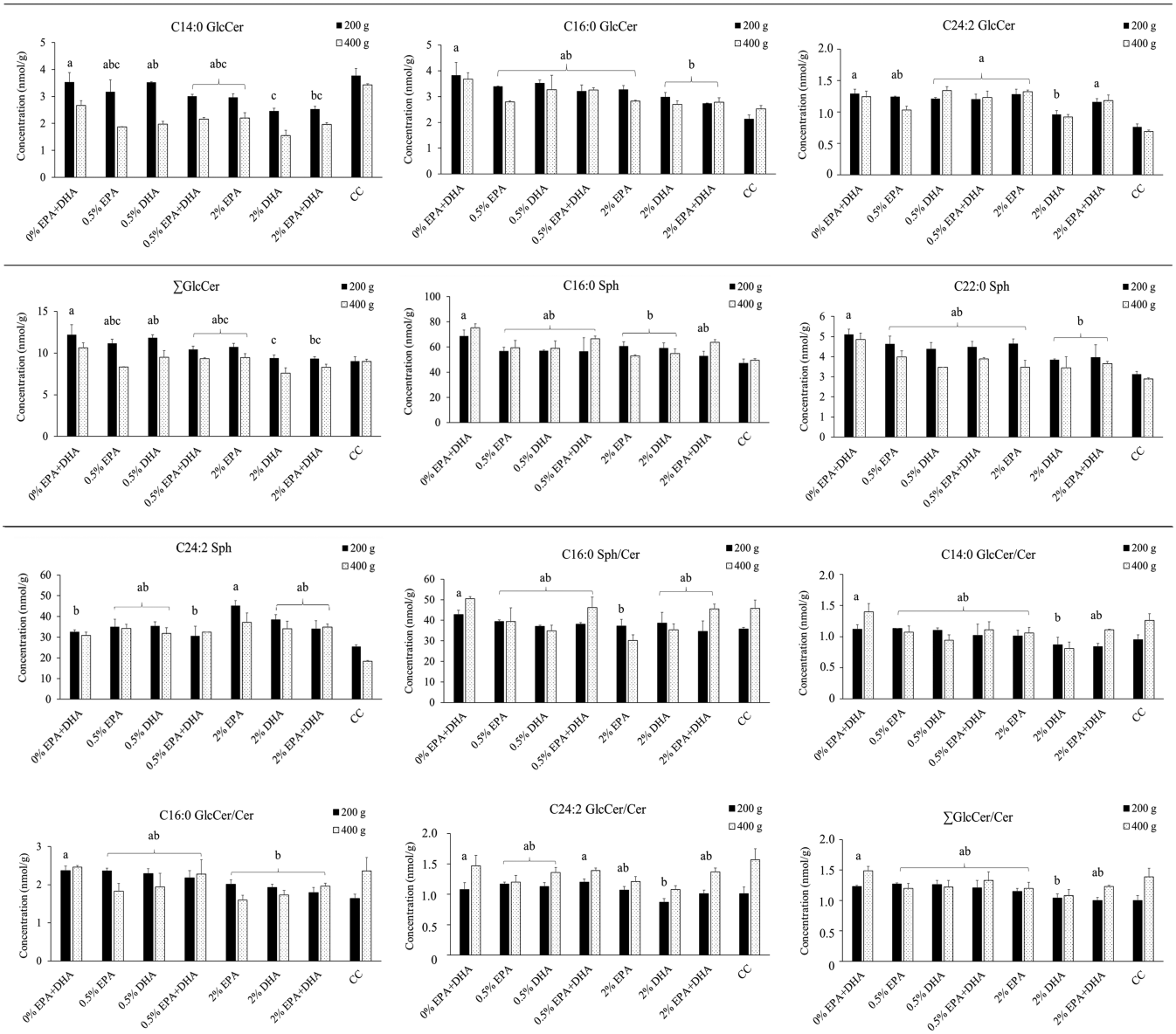

Figure 1. Absolute concentrations (nmol/g) of the important sphingolipids that were affected by diets in the skin of fish sampled at 200 and 400 g of body weight after feeding for 19 and 26 weeks, respectively (n = 2 for the 0.5% EPA and DHA groups, n = 3 for the other groups). Each statistical replicate originated from a pooled sample of skin from five fish. Different letters denote significant differences between dietary groups, except the commercial-type control (CC, $P < 0.05$). Cer, ceramide; GlcCer, glucosyl-ceramide; Sa, sphinganine; So, sphingosine; Sph, sphingomyelin.

using Hotelling's T^2 (95% confidence internal, CI) and DModX (95% CI). The PCA loading plots were used to identify the important metabolites that could distinguish groups.

All values are presented as means ± standard errors of the means (SE).

3. RESULTS

3.1. Sphingolipidomics in Skin. In order to study the effects of diet on sphingolipid metabolism in fish skin, five types of Cer (C14:0 Cer, C16:0 Cer, C18:0 Cer, C24:1 Cer, and C24:2 Cer), two types of So (d18:1 So and d20:1 So), two types of Sa (d18:0 Sa and d20:0 Sa), five types of GlcCer (C14:0 GlcCer, C16:0 GlcCer, C18:0 GlcCer, C24:1 GlcCer, and

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

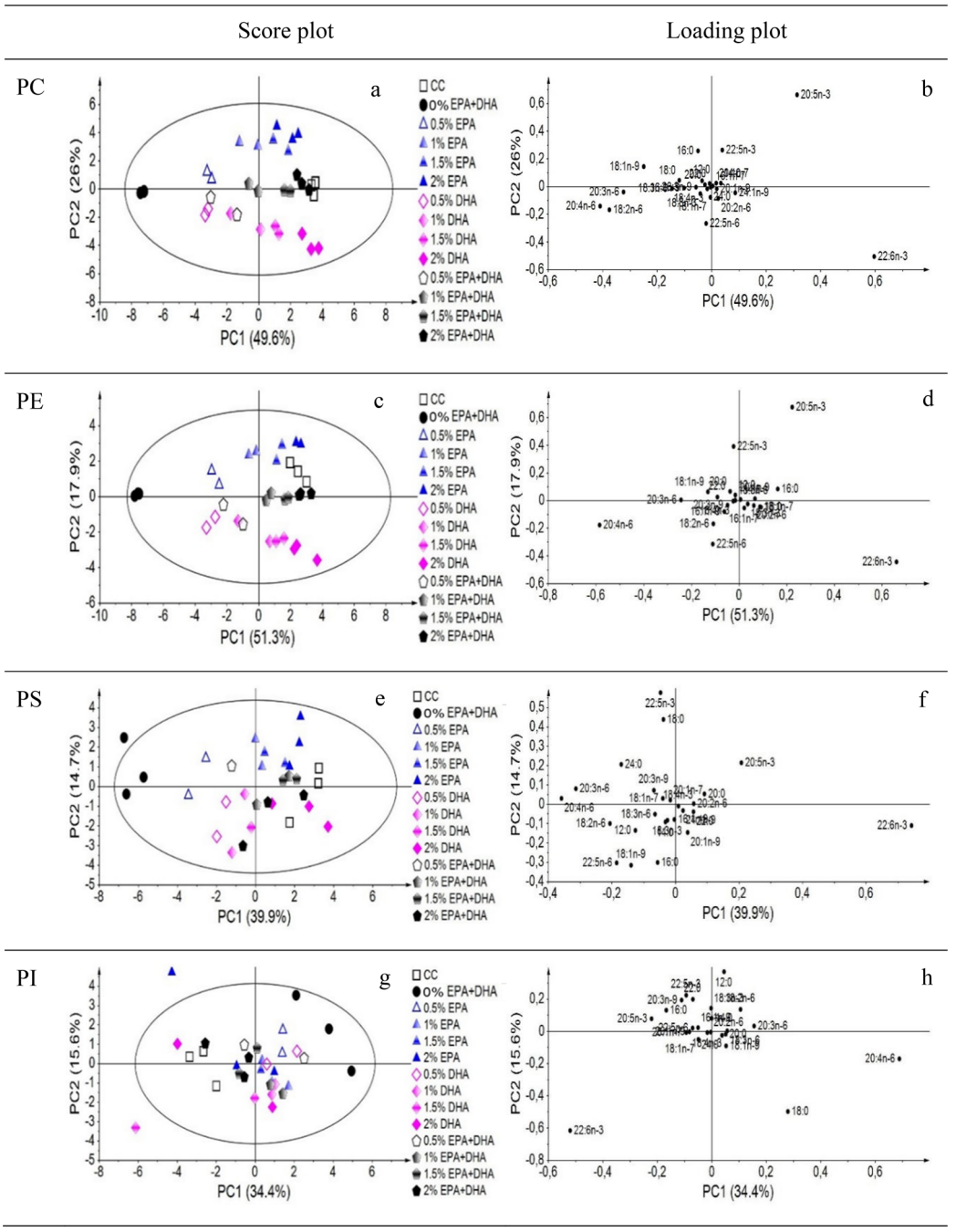

Figure 2. Principal-component-analysis (PCA) score plots and loading plots created with the fatty acid profile data for different glycerol-phospholipid fractions in skin samples of 400 g fish fed different diets for 26 weeks. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol. (a) PCA score plot and (b) PCA loading plot for the PC fraction (no outliers). The PCA model was established using three principal components (R^2X = 83.6%, Q^2 = 60.9%). The first (PC1) and second principal components (PC2) explained 49.6 and 26.0% of the data variation, respectively. (c) PCA score plot and (d) PCA loading plot for the PE fraction (one outlier from 2.0% EPA). The PCA model was established using two principal components (R^2X = 69.2%, Q^2 = 50.5%). PC1 and PC2 explained 51.3 and 17.9% of the data variation, respectively. (e) PCA score plot and (f) PCA loading plot for the PS fraction (one outlier from 0.5% EPA+DHA and one outlier from 1.5% DHA). The PCA model was established using six principal components (R^2X = 86.8%, Q^2 = 10.9%). PC1 and PC2 explained 39.9 and 14.7% of the data variation, respectively. (g) PCA score plot and (h) PCA loading plot for the PI fraction (one outlier from 2.0% DHA). The PCA model was established using two principal components (R^2X = 50.0%, Q^2 = 3.67%), with 34.4 and 15.6% of the data variation explained by PC1 and PC2, respectively. NC in the figures refers to the 0% EPA+DHA dietary group.

C24:2 GlcCer), and eight types of Sph (C14:0 Sph, C16:0 Sph, C18:0 Sph, C20:2 Sph, C22:0 Sph, C22:1 Sph, C24:1 Sph, and C24:2 Sph) were qualified and quantified using LC-ESI-QTOF MS (Table 2). Additionally, the sum of Cer (∑Cer), sum of So (∑So), sum of Sa (∑Sa), sum of GlcCer (∑GlcCer), ratio of Sph/Cer, and ratio of GlcCer/Cer were calculated (Table S2). The sphingolipids that were significantly affected by diets were plotted in Figure 1.

Comparing the 0% EPA+DHA group with the 0.5% EPA and DHA groups, there were no differences in sphingolipid

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

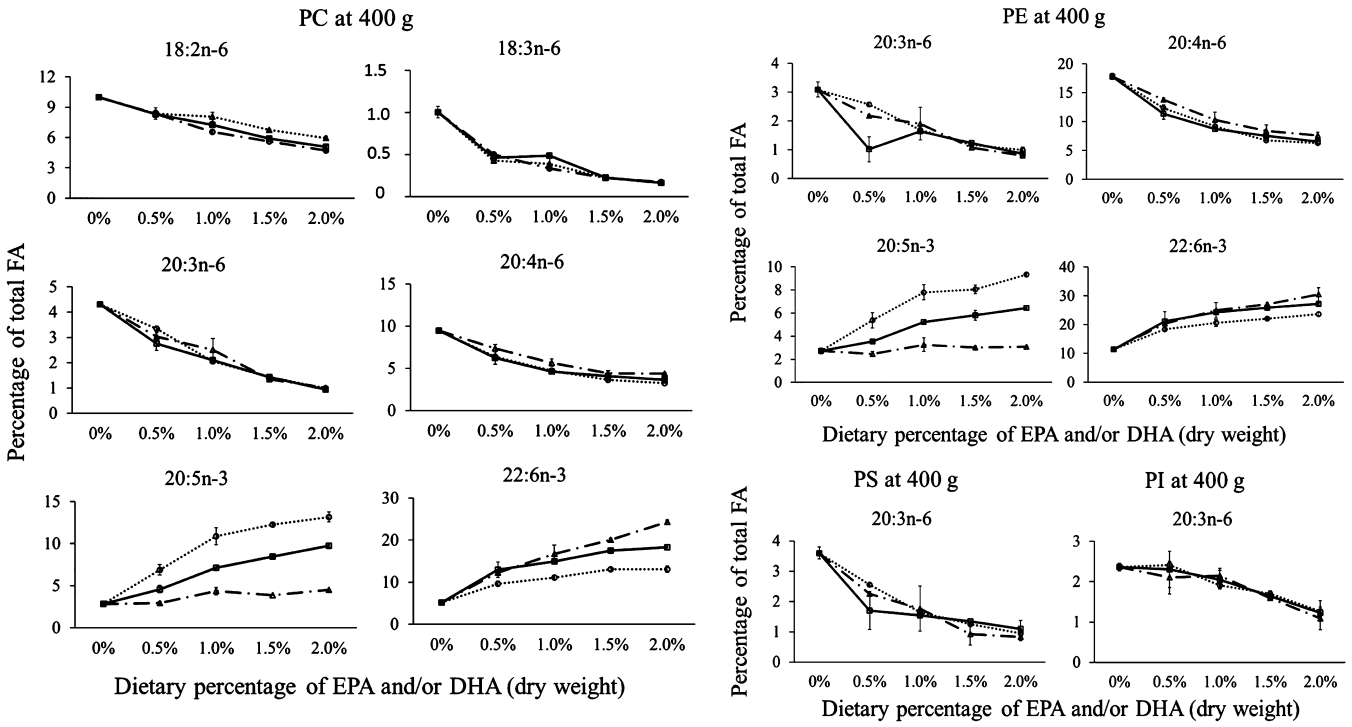

Figure 3. Compositions of the important fatty acids (FA, % of total FA) in skin phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) fractions of fish fed the experimental diets for 26 weeks (400 g weights; means ± SE; $n = 3$ for the CC and 0 and 2.0% EPA and DHA groups, $n = 2$ for the other groups). Each statistical replicate originated from a pooled sample of skin from five fish. EPA group, ○; DHA group, △; EPA+DHA group, □.

concentrations except for C18:0 Sph in 0.5% DHA. Comparing the 0% EPA+DHA group with the 2.0% groups indicated a decreased concentration of C16:0 Sph, decreased C16:0 Sph/Cer and C16:0 GlcCer/Cer values, and an increased concentration of C24:2 Sph in the 2.0% EPA group; reduced concentrations of C14:0 GlcCer, C16:0 GlcCer, C24:2 GlcCer, ∑GlcCer, C16:0 Sph, and C22:0 Sph and reduced C14:0 GlcCer/Cer, C16:0 GlcCer/Cer, C24:2 GlcCer/Cer, and ∑GlcCer/Cer values in the 2.0% DHA group; and lower concentrations of C14:0 GlcCer, C16:0 GlcCer, ∑GlcCer, and C22:0 Sph and a lower C16:0 GlcCer/Cer value in the 2.0% EPA+DHA group (Figure 1 and Table S2). Additionally, with increasing levels of dietary EPA and DHA, the concentrations of metabolites, including C16:0 GlcCer, C24:2 GlcCer, ∑GlcCer, C16:0 Sph, and C22:0 Sph, decreased gradually to levels close to those in the CC group (Figure 1).

Compared with those in the experimental groups, fish fed the CC diet had significantly lower concentrations of many sphingolipids, including C18:0 and C24:2 Cer; d18:1 So; ∑So; d18:0 and d20:0 Sa; ∑Sa; C16:0, C18:0, C24:1, and C24:2 GlcCer; ∑GlcCer; and C18:0, C22:0, and C24:0 Sph, but they had higher amounts of C14:0 Cer, C14:0 GlcCer, C14:0 Sph, and C20:2 Sph than the other experimental groups (Table S2, the statistical results including CC are not shown).

Effects of sampling time (at 200 and 400 g after feeding for 19 and 26 weeks, respectively) were observed for all types of sphingolipids (Table S2). Fish with average weights of 400 g had lower concentrations of metabolites (C14:0 Cer, C24:1 Cer, C24:2 Cer, ∑Cer, d20:1 So, d18:0 Sa, ∑Sa, C14:0 GlcCer, C24:1 GlcCer, ∑GlcCer, C14:0 Sph, C22:0 Sph, C22:1 Sph, C24:1 Sph, and ∑Sph) than fish weighing 200 g.

3.2. Fatty Acid Composition of Glycerol-Phospholipids in Skin. The dietary effects on FA composition were investigated in each GPL subclass using PCA and ANOVA data analysis. The dietary influences were more pronounced at 400 g (Figures 2 and 3 and Table S3) than at 200 g (Figure S1 and Table S4).

Overall, general separation was observed for the dietary groups in all the GPL subfractions but particularly for the PC fraction (Figure 2a,b). In contrast, the dietary groups were difficult to distinguish in the score plots of PS and PI, indicating that the FA composition in PS and PI was less affected by diet (Figure 2e–h).

Generally, the 0% EPA+DHA samples were clearly separated from the other groups in the PCA score plots at 400 g (Figure 2). The 0% EPA+DHA samples were characterized by higher proportions of n-6 FA, such as 18:2n-6 in the PC and PS fractions; 18:3n-6 in the PC fraction; 20:3n-6 in the PC, PE, and PS fractions; 20:4n-6 in all the GPL fractions; and 22:5n-6 in the PE fraction, and by lower levels of n-3 FA, such as 20:2n-3 and 20:5n-3 in the PC fraction and 22:6n-3 in all the GPL fractions (Figure 2). The FA profile of the CC samples was close to that of the 2.0% EPA+DHA samples, which was characterized by higher percentages of n-3 FA, such as 20:5n-3 and 22:6n-3 in the PC, PS, and PI fractions (Figure 2). Apart from these differences, the fish skin from the EPA groups had more n-3 FA (20:5n-3 and 22:5n-3) in the PC, PE, and PS fractions, and the skin from the DHA groups had more 22:5n-6 and 22:6n-3 in the PC and PE fractions (Figure 2).

With increases in the dietary levels of EPA and DHA from 0.5 to 2.0%, the sample score points shifted gradually along the horizon axis of the PCA score plots, offsetting from 0% EPA +DHA to CC (Figure 2). According to the univariate results at 400 g, with increasing dietary levels of EPA and DHA, the relative distributions of 20:5n-3 and 22:6n-3 increased in the PC fraction (in all groups and in the DHA and EPA+DHA groups,

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

respectively) and in the PE fraction (in the EPA and EPA+DHA groups and in all groups, respectively). Moreover, with increasing levels of EPA and DHA in the diet, there were declines in the proportions of 20:3n-6 in all GPL fractions, 20:4n-6 in the PC and PE fractions, and 18:2n-6 and 18:3n-6 in the PC fraction (Figure 3 and Table S3).

3.3. Histological Parameters of Fish Skin. There were no statistically significant differences in epidermal thickness (mean 30.8 μm) or numbers of goblet cells per 100 μm (average of 3.7) among the 0, 1.0, and 2.0% EPA and DHA groups (Table 2).

4. DISCUSSION

Dietary Effects on Sphingolipids in Salmon Skin. The sphingolipids GlcCer and Sph act as a reservoir for the production of Cer, which is essential for skin-barrier function in mammals.[23] A systemic anti-inflammatory effect of dietary GlcCer on skin diseases has been shown,[36,37] and up-regulation in the levels of GlcCer and Cer has been observed in cells and tissues in response to skin disorders and stressors.[38,39] In the present study, no EPA- or DHA-induced changes in the absolute content of Cer in fish skin were detected, but with declining levels of dietary EPA and DHA, the concentrations of several GlcCer and Sph in fish skin gradually increased, such as C16:0 GlcCer, C24:2 GlcCer, ∑GlcCer, C16:0 Sph, and C22:0 Sph. This implies that a reduction in dietary EPA and DHA can lead to an interruption in the sphingolipidome and possibly the barrier function of fish skin. This hypothesis was further confirmed by the increased ratios of Sph/Cer and GlcCer/Cer observed in the fish group fed a diet devoid of EPA and DHA, because it was shown that an increased ratio of Sph/Cer in skin negatively influenced the barrier function and microstructure of human skin.[25] Similar effects of LC-PUFA on the sphingolipidome have been observed in the hippocampus of aged rats, where dietary EPA and the EPA metabolite docosapentaenoic acid (DPA) exerted neuroprotective effects by reducing activation of sphingomyelinase, ceramidase, and sphingosine kinase, thereby down-regulating the generation of C16- and C18-Cer and increasing the ratios of sphingosine-1-phosphate (S1P)/Cer, including S1P/C16:0 Cer, S1P/C18:0 Cer, and S1P/C20:0 Cer.[31]

Furthermore, significant modifications in the concentrations of sphingolipids, such as C14:0 GlcCer, C16:0 GlcCer, ∑GlcCer, and C22:0 Sph, were mainly found in the 2.0% DHA and 2.0% EPA+DHA groups. This suggests that dietary DHA has stronger impacts in changing sphingolipid metabolism than EPA. Several other studies have shown that in some fish species, DHA is more efficient than EPA in increasing growth and survival rates,[40,41] but such effects were not seen in the current trial.[12] However, we observed that compared with EPA supplementation, dietary DHA supplementation had stronger effects on supporting normal intestinal structure and alleviating deficiency symptoms, such as cytoplasm packed with large or foamy vacuoles and swollen enterocytes in the intestine.[12]

Evidence has consistently shown that Cer and other sphingolipids act as signal molecules that play an important role in mediating cellular responses to stressors, such as infectious agents, toxins, and nutrient deprivation.[39] Stress leads to an accumulation of Cer in cells and tissues, which could promote apoptotic, inflammatory, and growth-inhibitory responses, for instance through disrupting the function of the mitochondrial respiratory chain and stimulating an increase in reactive-oxygen-species production by mitochondria.[31,39] Therefore, the higher contents of many sphingolipids (except C14:0 types) in the fish fed the experimental diets than in the fish fed the CC diet in the present study suggest that compared with the CC group, fish from the experimental groups were exposed to more nutritional stress. This could be due to the different dietary ingredients between CC and the experimental diets, such as the fishmeal and fish oil in CC. Furthermore, it has been shown that the cellular function of Cer depends on the type of FA attached to the sphingoid base.[39] This may explain the much higher content of C14:0 sphingolipids in the CC group.

Dietary Effects on FA Composition in GPL in Salmon Skin. Effects of dietary EPA and DHA on FA composition in GPL subclasses were also observed. When the levels of dietary EPA and DHA declined, the percentages of n-3 FA, such as that of 22:6n-3, were markedly reduced in the GPL subfractions, while the proportions of n-6 FA, such as those 20:3n-6 and 20:4n-6, increased to compensate. This demonstrates that the FA composition in GPL in Atlantic salmon skin is strongly affected by diet, which is consistent with findings in rainbow trout.[29] Moreover, because of the inclusion of poultry oil and rapeseed oil as the base oil in the experimental diets, the highest content of 18:2n-6 was found in the 0% EPA+DHA diet. The increased accumulation of n-6 PUFA, such as 20:3n-6 and 20:4n-6 in the EPA- and DHA-deficient groups indicated increased desaturation and elongation of 18:2n-6 to longer-chain n-6 PUFA. A notable increase in Δ5-desaturase and Δ6-desaturase has been observed previously in the liver and blood of Atlantic salmon fed EFA-deficient diets.[1,2,12] Some studies have found that dietary EFA deficiency increases the levels of n-9 PUFA, especially 20:3n-9, in the skin of rainbow trout and in the organs and plasma of Atlantic salmon.[1,2,13,29] However, there was no significant change in 20:3n-9 composition in the present study, although an increasing tendency in PC was seen with reduced levels of dietary EPA and DHA. This is probably because the EFA-deficient diets in previous studies contained little n-3 or n-6 FA, so n-9 FA was desaturated and elongated.

The dietary effects on FA composition were more distinct in the PC and PE fractions than in the PS and PI fractions. According to the univariate results, for instance, the significant diet-induced modifications of proportions of 20:5n-3 and 22:6n-3 only occurred in the PC and PE fractions. This indicates that PS and PI are more conserved and resistant to dietary FA changes, which may be caused by a shift from triacylglycerol to PS and PI through the incorporation of FA hydrolyzed from TAG into PS and PI.[1] Moreover, with increasing dietary EPA and DHA, there were significantly reduced levels of 18:1n-9 in the skin PC fraction but not in the other GPL fractions. This may be caused by the experimental diets in which EPA and DHA oils were replaced with rapeseed oil containing high levels of 18:1n-9.

The changes in FA composition in skin GPL fractions could also be implicated in fish-skin health. A study on guinea pigs by Miller et al.[42] showed that dietary supplementation with fish oil resulted in the incorporation of EPA and DHA into epidermal GPL and increased epidermal levels of PUFA-derived 15-lipoxygenase products (eicosanoids), which improved chronic inflammatory skin disorders. Furthermore, Sph in skin could reduce eicosanoid production from GPL through the inhibition of cytosolic phospholipase A_2 (cPLA2α)-binding to GPL.[23] Thus, the decreased concentrations of Sph in skin and the greater EPA and DHA incorporation into GPL in fish fed increased levels of dietary EPA and DHA observed in our study suggest that dietary EPA and DHA might improve the anti-

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

inflammatory and protective-barrier capacities of fish skin by regulating the production of eicosanoids.

However, there were no significant differences among the experimental groups in terms of epidermal thickness or mucus-cell density, possibly because of the great variation in these epidermal histological parameters among individual fish ($n = 10$ fish per dietary treatment). More individual samples are probably needed to detect significant changes in these parameters.

Time Course of Changes. With increasing length of the experimental trial (19 and 26 weeks), the modifications in FA composition in skin GPL subclasses became more noticeable. This is consistent with previous findings on the changes in FA composition over time in the liver and blood of Atlantic salmon.[1] The absolute concentrations of most sphingolipids were significantly lower in skin samples of fish weighing 400 g than in those weighing 200 g, which may be due to the increased weights of other components, such as scales and collagen, in the skin samples.

In conclusion, reductions in dietary EPA and DHA modified the phospholipid profile in the skin of Atlantic salmon, especially the concentrations of the sphingolipids GlcCer and Sph and the relative contents of n-3 and n-6 FA in the GPL fractions. These changes could affect fish-skin health, although we found no significant effects on epidermal thickness or mucus-cell density, because of the small numbers of samples. The current results provide new insights into the importance of dietary EPA and DHA for membrane lipid composition in fish skin. In future work, it would be interesting to identify the functional mechanisms of GPL and sphingolipids in fish-skin health, such as those of their anti-inflammatory and immune effects, and the function of membrane lipids in other fish barrier tissues in contact with the external environment, such as gills.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b02886.

Principal-component-analysis (PCA) score plots and loading plots created with the fatty acid profile data for different phospholipid fractions in skin samples, feed composition, and detailed results of sphingolipids and FA composition of GPL in salmon skin (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +46 18 6720 11. E-mail: cheng-ken@live.cn.

ORCID

Ken Cheng: 0000-0003-3910-0220

Funding

This work was supported by the Norwegian Research Council (grant number NFR 224913). The authors also thank the China Scholarship Council for financial support.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

CC, commercial-like control diet; Cer, ceramide; CI, confidence internal; cPLA2α, cytosolic phospholipase A_2; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EFA, essential fatty acids; ELSD, evaporative-light-scattering detection; EPA, eicosapentaenoic acid; EPA+DHA, dietary group given a 1:1 mixture of EPA and DHA; ESI-QTOF MS, electrospray ionization−quadropole time-of-flight mass spectrometry; FA, fatty acid; FAME, fatty acid methyl ester; GlcCer, glucosylceramide; GPL, glycerol-phospholipid; HILIC, hydrophilic-interaction chromatography; HPLC, high-pressure liquid chromatography; LC-MS, liquid chromatography−mass spectrometry; LC-PUFA, long-chain polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; PCA, principal-component analysis; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; S1P, sphingosine-1-phosphate; Sa, sphinganine; SE, standard error of the mean; So, sphingosine; Sph, sphingomyelin; TLC, thin-layer chromatography; UV, ultraviolet

■ REFERENCES

(1) Ruyter, B.; Rosjo, C.; Einen, O.; Thomassen, M. S. Essential Fatty Acids in Atlantic Salmon: Time Course of Changes in Fatty Acid Composition of Liver, Blood and Carcass Induced by a Diet Deficient in n-3 and n-6 Fatty Acids. *Aquacult. Nutr.* **2000**, *6*, 109−117.

(2) Ruyter, B.; Rosjo, C.; Einen, O.; Thomassen, M. S. Essential Fatty Acids in Atlantic Salmon: Effects of Increasing Dietary Doses of n-6 and n-3 Fatty Acids on Growth, Survival and Fatty Acid Composition of Liver, Blood and Carcass. *Aquacult. Nutr.* **2000**, *6*, 119−127.

(3) Ruyter, B.; Røsjø, C.; MÅsøval, K.; Einen, O.; Thomassen, M. S. Influence of Dietary N-3 Fatty Acids on the Desaturation and Elongation of [1-^{14}C] 18:2n-6 and [1-^{14}C] 18:3n-3 in Atlantic Salmon Hepatocytes. *Fish Physiol. Biochem.* **2000**, *23*, 151−158.

(4) Naylor, R. L. R. L.; Hardy, R. W.; Bureau, D. P.; Chiu, A.; Elliott, M.; Farrell, A. P.; Forster, I.; Gatlin, D. M.; Goldburg, R. J.; Hua, K.; et al. Feeding Aquaculture in an Era of Finite Resources. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 15103−15110.

(5) Ytrestøyl, T.; Aas, T. S.; Åsgård, T. Utilisation of Feed Resources in Production of Atlantic Salmon (*Salmo Salar*) in Norway. *Aquaculture* **2015**, *448*, 365−374.

(6) Olsen, Y. Resources for Fish Feed in Future Mariculture. *Aquac. Environ. Interact.* **2011**, *1*, 187−200.

(7) Sissener, N. H.; Sanden, M.; Krogdahl, Å.; Bakke, A.-M.; Johannessen, L. E.; Hemre, G.-I. Genetically Modified Plants as Fish Feed Ingredients. *Can. J. Fish. Aquat. Sci.* **2011**, *68*, 563−574.

(8) Cheng, K.; Müllner, E.; Moazzami, A. A.; Carlberg, H.; Brännäs, E.; Pickova, J. Metabolomics Approach to Evaluate a Baltic Sea Sourced Diet for Cultured Arctic Char (*Salvelinus Alpinus* L.). *J. Agric. Food Chem.* **2017**, *65*, 5083−5090.

(9) Sprague, M.; Dick, J. R.; Tocher, D. R. Impact of Sustainable Feeds on Omega-3 Long-Chain Fatty Acid Levels in Farmed Atlantic Salmon, 2006−2015. *Sci. Rep.* **2016**, *6*, 21892.

(10) Ruyter, B.; Thomassen, M. S. Metabolism of n-3 and n-6 Fatty Acids in Atlantic Salmon Liver: Stimulation by Essential Fatty Acid Deficiency. *Lipids* **1999**, *34*, 1167−1176.

(11) Bou, M.; Berge, G. M.; Baeverfjord, G.; Sigholt, T.; Østbye, T.-K.; Ruyter, B. Low Levels of Very-Long-Chain n-3 PUFA in Atlantic Salmon (*Salmo Salar*) Diet Reduce Fish Robustness under Challenging Conditions in Sea Cages. *J. Nutr. Sci.* **2017**, *6*, 1−14.

(12) Bou, M.; Berge, G. M.; Baeverfjord, G.; Sigholt, T.; Østbye, T.-K.; Romarheim, O. H.; Hatlen, B.; Leeuwis, R.; Venegas, C.; Ruyter, B. Requirements of n-3 Very Long-Chain PUFA in Atlantic Salmon (*Salmo Salar* L): Effects of Different Dietary Levels of EPA and DHA on Fish Performance and Tissue Composition and Integrity. *Br. J. Nutr.* **2017**, *117*, 30−47.

(13) Thomassen, M. S.; Bou, M.; Røsjø, C.; Ruyter, B. Organ and Phospholipid Class Fatty Acid Specificity in Response to Dietary Depletion of Essential n-3 Fatty Acids in Atlantic Salmon (*Salmo Salar* L.). *Aquacult. Nutr.* **2017**, *23*, 433−443.

(14) Cheng, K.; Wagner, L.; Moazzami, A. A.; Gomez-Requeni, P.; Schiller Vestergren, A.; Brannas, E.; Pickova, J.; Trattner, S. Decontaminated Fishmeal and Fish Oil from the Baltic Sea are

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Promising Feed Sources for Arctic Char (*Salvelinus Alpinus* L.)-Studies of Flesh Lipid Quality and Metabolic Profile. *Eur. J. Lipid Sci. Technol.* **2016**, *118*, 862−873.
(15) Cheng, K.; Wagner, L.; Pickova, J.; Moazzami, A. A. NMR-Based Metabolomics Reveals Compartmental Metabolic Heterogeneity in Liver of Arctic Char (*Salvelinus Alpinus* L.). *Can. J. Zool.* **2016**, *94*, 665−669.
(16) Glover, C. N.; Bucking, C.; Wood, C. M. The Skin of Fish as a Transport Epithelium: A Review. *J. Comp. Physiol., B* **2013**, *183* (7), 877−891.
(17) Rakers, S.; Niklasson, L.; Steinhagen, D.; Kruse, C.; Schauber, J.; Sundell, K.; Paus, R. Antimicrobial Peptides (AMPs) from Fish Epidermis: Perspectives for Investigative Dermatology. *J. Invest. Dermatol.* **2013**, *133*, 1140−1149.
(18) Lewis, R. W. Fish Cutaneous Mucus: A New Source of Skin Surface Lipid. *Lipids* **1970**, *5*, 947.
(19) Brinchmann, M. F. Immune Relevant Molecules Identified in the Skin Mucus of Fish Using-Omics Technologies. *Mol. BioSyst.* **2016**, *12*, 2056−2063.
(20) Fast, M. D.; Sims, D. E.; Burka, J. F.; Mustafa, A.; Ross, N. W. Skin Morphology and Humoral Non-Specific Defence Parameters of Mucus and Plasma in Rainbow Trout, Coho and Atlantic Salmon. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **2002**, *132*, 645−657.
(21) Subramanian, S.; MacKinnon, S. L.; Ross, N. W. A Comparative Study on Innate Immune Parameters in the Epidermal Mucus of Various Fish Species. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **2007**, *148*, 256−263.
(22) Buchmann, K.; Bresciani, J. Microenvironment of Gyrodactylus Derjavini on Rainbow Trout *Oncorhynchus Mykiss*: Association between Mucous Cell Density in Skin and Site Selection. *Parasitol. Res.* **1997**, *84*, 17−24.
(23) Kendall, A. C.; Nicolaou, A. Bioactive Lipid Mediators in Skin Inflammation and Immunity. *Prog. Lipid Res.* **2013**, *52*, 141−164.
(24) Feingold, K. R. The Role of Epidermal Lipids in Cutaneous Permeability Barrier Homeostasis. *J. Lipid Res.* **2007**, *48*, 2531−2546.
(25) Pullmannová, P.; Staňková, K.; Pospíšilová, M.; Školová, B.; Zbytovská, J.; Vávrová, K. Effects of Sphingomyelin/Ceramide Ratio on the Permeability and Microstructure of Model Stratum Corneum Lipid Membranes. *Biochim. Biophys. Acta, Biomembr.* **2014**, *1838*, 2115−2126.
(26) Duan, J.; Sugawara, T.; Hirata, T. Rapid Quantitative Analysis of Sphingolipids in Seafood Using HPLC with Evaporative Light-Scattering Detection: Its Application in Tissue Distribution of Sphingolipids in Fish. *J. Oleo Sci.* **2010**, *59*, 509−513.
(27) Tocher, D. R.; Bendiksen, E. Å.; Campbell, P. J.; Bell, J. G. The Role of Phospholipids in Nutrition and Metabolism of Teleost Fish. *Aquaculture* **2008**, *280*, 21−34.
(28) Yang, B.; Kalimo, K. O.; Tahvonen, R. L.; Mattila, L. M.; Katajisto, J. K.; Kallio, H. P. Effect of Dietary Supplementation with Sea Buckthorn (*Hippophae Rhamnoides*) Seed and Pulp Oils on the Fatty Acid Composition of Skin Glycerophospholipids of Patients with Atopic Dermatitis. *J. Nutr. Biochem.* **2000**, *11*, 338−340.
(29) Ghioni, C.; Bell, J. G.; Bell, M. V.; Sargent, J. R. Fatty Acid Composition, Eicosanoid Production and Permeability in Skin Tissues of Rainbow Trout (*Oncorhynchus Mykiss*) Fed a Control or an Essential Fatty Acid Deficient Diet. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1997**, *56* (6), 479−489.
(30) Schäfer, L.; Kragballe, K. Abnormalities in Epidermal Lipid Metabolism in Patients with Atopic Dermatitis. *J. Invest. Dermatol.* **1991**, *96*, 10−15.
(31) Kelly, L.; Grehan, B.; Chiesa, A. D.; O'Mara, S. M.; Downer, E.; Sahyoun, G.; Massey, K. A.; Nicolaou, A.; Lynch, M. A. The Polyunsaturated Fatty Acids, EPA and DPA Exert a Protective Effect in the Hippocampus of the Aged Rat. *Neurobiol. Aging* **2011**, *32*, 2318.e1−2318.e15.
(32) Shaner, R. L.; Allegood, J. C.; Park, H.; Wang, E.; Kelly, S.; Haynes, C. A.; Sullards, M. C.; Merrill, A. H. Quantitative Analysis of Sphingolipids for Lipidomics Using Triple Quadrupole and Quadrupole Linear Ion Trap Mass Spectrometers. *J. Lipid Res.* **2009**, *50*, 1692−1707.
(33) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.
(34) Thomassen, M. S.; Bou, M.; Røsjø, C.; Ruyter, B. Organ and Phospholipid Class Fatty Acid Specificity in Response to Dietary Depletion of Essential n-3 Fatty Acids in Atlantic Salmon (*Salmo Salar* L.). *Aquacult. Nutr.* **2017**, *23*, 433−443.
(35) Mason, M. E.; Waller, G. R. Dimethoxypropane Induced Transesterification of Fats and Oils in Preparation of Methyl Esters for Gas Chromatographic Analysis. *Anal. Chem.* **1964**, *36*, 583−586.
(36) Yeom, M.; Kim, S. H.; Lee, B.; Han, J. J.; Chung, G. H.; Choi, H. D.; Lee, H.; Hahm, D. H. Oral Administration of Glucosylceramide Ameliorates Inflammatory Dry-Skin Condition in Chronic Oxazolone-Induced Irritant Contact Dermatitis in the Mouse Ear. *J. Dermatol. Sci.* **2012**, *67*, 101−110.
(37) Tsuji, K.; Mitsutake, S.; Ishikawa, J.; Takagi, Y.; Akiyama, M.; Shimizu, H.; Tomiyama, T.; Igarashi, Y. Dietary Glucosylceramide Improves Skin Barrier Function in Hairless Mice. *J. Dermatol. Sci.* **2006**, *44*, 101−107.
(38) Nagai, K. Alteration of Fatty Acid Molecular Species in Ceramide and Glucosylceramide under Heat Stress and Expression of Sphingolipid-Related Genes. *Adv. Biol. Chem.* **2011**, *1*, 35−48.
(39) Messner, M. C.; Cabot, M. C. Glucosylceramide in Humans. *Sphingolipids as Signaling and Regulatory Molecules*; Chalfant, C., Maurizio, D. P., Eds.; Springer: New York, 2010; pp 156−164.
(40) Kanazawa, A. Effects of Docosahexaenoic Acid and Phospholipids on Stress Tolerance of Fish. *Aquaculture* **1997**, *155*, 129−134.
(41) Watanabe, T. Importance of Docosahexaenoic Acid in Marine Larval Fish. *J. World Aquacult. Soc.* **1993**, *24*, 152−161.
(42) Miller, C. C.; Tang, W.; Ziboh, V. A.; Fletcher, M. P. Dietary Supplementation with Ethyl Ester Concentrates of Fish Oil (n-3) and Borage Oil (n-6) Polyunsaturated Fatty Acids Induces Epidermal Generation of Local Putative Anti-Inflammatory Metabolites. *J. Invest. Dermatol.* **1991**, *96*, 98−103.

DOI: 10.1021/acs.jafc.8b02886
J. Agric. Food Chem. XXXX, XXX, XXX−XXX