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Marine By-Products Tested as Feed for Almaco Jack *Seriola rivoliana* and Their Effect on Fatty Acids and Sterols in Different Tissues

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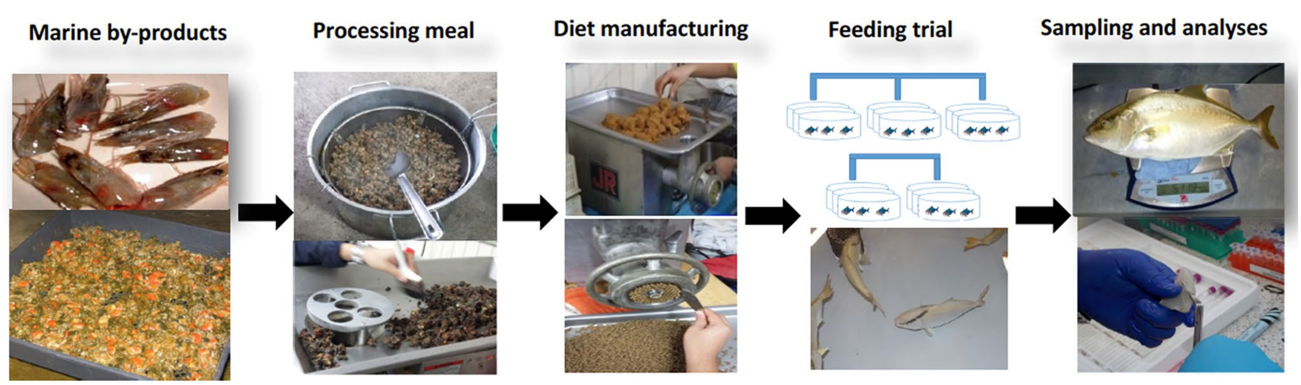
Abstract

Marine by-products can compose up to 70% of the total weight of products from fisheries, most of which are discarded. However, these by-products are rich in highly unsaturated fatty acids that are not synthetized by most marine animals produced by aquaculture. Here, we used three marine by-products (shrimp head, Catarina scallop viscera, and Pen shell viscera) to produce lipid-rich (72.9–144.6 g/kg) meals which were used to partially substitute commercial fishmeal (FM) on feeds that were used to grow Almaco Jack (*Seriola rivoliana*) juveniles for 10 weeks. The content of 20:5n-3 and 22:6n-3 in tissues of fish fed shrimp and Pen shell presented values similar to controls, but the former had a better effect on growth, lipid, and phytosterols levels. Catarina meal had lower concentration of 20:4n-6 and 22:6n-3 in feed but promoted higher proportion of 20:4n-6 in muscle and 22:6n-3 in liver, indicating a selective conservation in relation to other fatty acids. Catarina meal contained traces of 18:5n-3 (0.02 g/kg) indicating that scallops probably ingested dinoflagellates; after testing, phycotoxins like okadaic acid (OA) and dinophysistoxin 1 (DTX1) were detected by mouse bioassay, by lateral flow immunochromatography, and quantified by HPLC–MS/MS. The presence of these toxins at the detected concentrations (OA: 27.64 µg/g and DTX1: 10.31 µg/g) affected almaco jack juveniles, a setback that needs to be addressed before meal manufacturing from mollusks. Marine by-products rich in lipids can be used to reduce the use of FM in the diet, and their use improve the lipid content and growth compared to control diet with FM.

Graphical Abstract



Keywords Arachidonic acid · Docosahexaenoic acid · Eicosapentaenoic acid · Marine fish · Okadaic acid · Phytosterol

Statement of Novelty

Marine fisheries produce a substantial quantity of by-products, particularly viscera that are usually thrown into landfills or directly into the sea. However, they are very rich

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in nutrients, particularly in highly unsaturated fatty acids (HUFA), pigments, and other essential lipids that are needed for aquaculture of marine commercial species and that drive the price of the feed up. Lipids are very easily hydrolyzed and then oxidized during the traditional meal production process, which affects HUFA deposition in the muscle of the animals that are fed on these meals, and ultimately, human health. Here, we tested three meals made with a lipid-conservation processing in mind and compared different marine by-product meals to determine the effects on lipid composition of almaco jack juveniles.

Introduction

Fishmeal (FM) has been traditionally used as the main source of protein in fish aquaculture, but the steady decline in global fisheries and the higher demand for animal feed have drastically limited the availability of FM and has increased its cost [1]. Hence, the use of FM in aquafeeds has been gradually reduced by using alternative sources of proteins and lipids. Plant-based meals and by-products from terrestrial or marine animals derived from fisheries and aquaculture have been tested to replace FM [2]. Plant and terrestrial animals can deliver the necessary levels of proteins, but they mostly lack HUFA, which must be provided by fish oil, again relying on fisheries. Marine by-products are derived from waste of fisheries or aquaculture or even algae and other organisms that gather at the shore and can constitute a pollution problem. Of the 45,000 million tons of marine by-products produced per year from fisheries and aquaculture [3], part is used for human consumption in some countries, another part is used to produce chitosan and glucosamine, pigments, and other nutraceutical and pharmacological products, but most is still discarded either directly in the ocean or ditched near processing sites, generating pollution and health issues to local communities [4]. Marine by-products, composed of digestive gland/liver, brains, gonads, etc., are naturally rich in essential nutrients, such as HUFA, amino acids, vitamins, pigments, and minerals that are not synthetized by most marine organisms produced by aquaculture. While partial or total marine by-products substitution of FM has begun to be evaluated with mostly good results on growth and survival in diets for shrimp [5, 6] and marine finfish [2, 7–9], there are still several concerns that remain, mainly the quantity of by-product meal from individual sources that can be produced each year and be commercially available for inclusion in feeds, an adequate ratio of n-3 and n-6 HUFA, enough docosahexaenoic acid (22:6n-3, DHA), the presence of toxins, hormones, phytosterols or other nutrients in by-products that can be or not present in FM and that can affect survival and growth in organisms fed feeds made with these by-products. The process of producing meal from different by-products can also differ from one to the other, and their composition can affect the quality of the meal [10]. While shrimp accept more readily diversity in feed, fish, particularly carnivorous fish can be more squeamish. For example, in previous studies we found that shrimp *Litopenaeus vannamei* fed marine by-product meals in diets not only had a better growth and general performance compared to shrimp fed FM, but also actively sought the feeds made with by-product meals [6]. However, feeds made with a partial substitution of FM with similar by-products could, depending on the type of by-product, increase growth and feed palatability when given to almaco jack *Seriola rivoliana* juveniles, or be actively rejected, affecting their growth and hematological parameters [9]. Carnivorous fish with high growth rates, like *S. rivoliana,* require large amounts of essential amino acids and HUFA in the diet, and by-products might not be suppling enough for their accelerated growth, even if these by-products have more than enough HUFA for shrimp, or they might contain microalgae toxins that affect fish but not shrimp. Finally, the feeds not only have to promote fish growth, but preferably enrich the edible part of the fish or shrimp (muscle) with nutrients that are sought for human consumption, such as HUFA. Here we aimed at evaluating the lipid composition in muscle, liver, brain, and mesenteric fat of *S. rivoliana* juveniles fed diets containing Pen shell viscera*,* Catarina scallop viscera, and/or shrimp heads lipid-rich meals to assess the use of marine by-products as partial substitutes for FM in feeds.

Materials and Methods

Ingredients and Experimental Diets

Shrimp heads (*Litopenaeus stylirostris*), and viscera from Catarina scallop (*Argopecten ventricosus*) and Pen shell (*Atrina maura*) were collected from fishermen communities in Puerto Cancun, B.C.S. Mexico, and Puerto San Carlos, B.C.S. Mexico, respectively, packed in ice for transportation to CIBNOR, and stored at − 18 °C until processing. Meals were made according to the method described by Toyes-Vargas et al. [10]. Briefly, batches of 2 kg were submerged in 80 L of boiling water for 10 min. Cooked by-products were homogenized in a meat grinder, then placed in plastic trays and dried inside a forced–air oven at 60 °C for 24 h. The dried products were ground, totally strained through a 0.25 mm mesh sieve and stored in plastic bags under refrigeration (4 °C) until chemical analyses.

The proximate composition, gross energy, fatty acids, and sterols content in the ingredients used for the experimental diets are shown in Table 1. Five diets were prepared as described by Civera and Guillaume [11] and evaluated in a 60-day growth trial. The dietary treatments

Table 1 Proximate composition (g/kg dry matter), gross energy (MJ/kg), fatty acids (g/kg dry matter) and sterols (g/kg dry matter) content in the main ingredients used for the diets

	Fishmeal[a]	SPC[b]	Wheat meal[c]	Shrimp head meal	Catarina viscera meal	Pen shell viscera meal
Proximate composition						
Dry matter	939.4 ± 0.7	922.7 ± 1.3	880.9 ± 0.9	923.5 ± 1.2	947.2 ± 0.3	906.9 ± 0.9
Crude protein	699.3 ± 0.4	583.1 ± 0.8	129.3 ± 0.1	543.4 ± 1.1	577.8 ± 1.7	519.6 ± 0.9
Ether extract	61.2 ± 0.3	11.2 ± 0.5	10.6 ± 0.4	72.9 ± 1.2	144.6 ± 1.9	138.1 ± 1.4
Crude fiber	2.2 ± 0.5	31.6 ± 2.3	1.2 ± 0.5	75.4 ± 0.6	2.2 ± 0.6	1.5 ± 0.5
Ash	161.7 ± 0.2	31.3 ± 0.3	5.8 ± 0.3	186.5 ± 0.9	86.1 ± 0.3	76.5 ± 0.5
NFE[d]	15.0 ± 0.1	265.4 ± 1.8	734.0 ± 0.9	45.3 ± 1.9	136.5 ± 2.2	171.2 ± 1.6
Gross energy	18.5 ± 0.1	18.3 ± 0.1	15.5 ± 0.1	16.7 ± 0.1	21.5 ± 0.1	19.7 ± 0.1
Fatty acids						
16:0	8.5 ± 0.2	2.2 ± 0.04	1.0 ± 0.2	4.0 ± 0.02	10.0 ± 0.1	14.2 ± 1.0
18:0	2.1 ± 0.2	0.8 ± 0.03	0.1 ± 0.01	2.6 ± 0.1	2.9 ± 0.03	4.5 ± 0.3
16:1n-9	0.3 ± 0.0	ND	0.01 ± 0.0	0.4 ± 0.1	0.2 ± 0.01	ND
16:1n-7	1.4 ± 0.03	0.02 ± 0.0	0.01 ± 0.0	1.5 ± 0.02	3.3 ± 0.2	3.6 ± 0.3
18:1n-9	3.1 ± 0.1	2.0 ± 0.01	0.8 ± 0.1	2.8 ± 0.02	0.9 ± 0.2	2.0 ± 0.02
18:1n-7	1.1 ± 0.03	0.2 ± 0.0	0.1 ± 0.01	1.5 ± 0.02	1.1 ± 0.01	3.2 ± 0.2
18:2n-6	0.6 ± 0.01	7.3 ± 0.1	3.5 ± 0.5	0.4 ± 0.01	0.2 ± 0.0	0.7 ± 0.1
18:3n-3	0.4 ± 0.02	0.8 ± 0.01	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.02
18:4n-3	0.6 ± 0.02	ND	ND	0.1 ± 0.01	0.1 ± 0.01	1.2 ± 0.2
18:5n-3	ND	ND	ND	ND	0.02 ± 0.0	ND
20:4n-6	0.5 ± 0.01	ND	ND	2.4 ± 0.1	0.2 ± 0.02	1.4 ± 0.2
20:5n-3	.4 ± 0.1	ND	ND	4.0 ± 0.2	0.4 ± 0.02	12.6 ± 1.7
22:6n-3	11.5 ± 0.6	ND	ND	3.5 ± 0.2	0.4 ± 0.1	12.1 ± 1.7
SFA	13.2 ± 0.4	3.2 ± 0.1	1.2 ± 0.2	8.9 ± 0.1	17.3 ± 0.2	22.9 ± 1.5
MUFA	9.7 ± 0.6	2.5 ± 0.02	1.1 ± 0.2	9.4 ± 0.1	7.1 ± 0.5	13.4 ± 0.7
PUFA	18.6 ± 0.8	8.2 ± 0.1	3.7 ± 0.6	12.3 ± 0.6	1.7 ± 0.1	31.0 ± 4.2
HUFA	17.3 ± 0.7	0.04 ± 0.01	0.01 ± 0.0	11.2 ± 0.6	1.2 ± 0.05	29.6 ± 4.1
n-3/n-6	8.7 ± 0.4	0.1 ± 0.0	0.1 ± 0.0	2.3 ± 0.1	1.6 ± 0.03	7.8 ± 0.2
Sterols						
DHC	1.01 ± 0.3	ND	ND	2.0 ± 1.2	3.6 ± 1.5	3.0 ± 1.8
Cholesterol	16.7 ± 1.9	ND	ND	33.2 ± 17.7	15.9 ± 4.9	12.3 ± 6.9
Brassicasterol	0.9 ± 0.5	ND	ND	0.7 ± 0.3	8.3 ± 2.7	7.2 ± 4.2
Campesterol	ND	0.04 ± 0.02	0.21 ± 0.12	0.10 ± 0.8	4.7 ± 1.5	1.4 ± 0.8
Stigmasterol	ND	0.06 ± 0.03	ND	0.19 ± 0.8	7.1 ± 2.3	9.7 ± 5.7
β-Sitosterol		0.18 ± 0.10	0.53 ± 0.35	0.14 ± 0.11	4.1 ± 1.3	2.9 ± 1.2
Fucosterol	ND	0.02 ± 0.01	0.10 ± 0.07	ND	1.2 ± 0.4	1.0 ± 0.8

Results are expressed as means ± SD, n = 3

ND not detected

[a]Monterey sardine (Conservera San Carlos, Puerto San Carlos, B.C.S., México)

[b]Soybean protein concentrate (Promotora Industrial Acuasistemas, S.A. de C.V. La Paz, B.C.S., México

[c]Wheat meal (Central de Abastos de La Paz, B.C.S., México)

[d]Nitrogen-free-extract (NFE) = 1000 – (moisture g/kg + crude protein g/kg + ether extract g/kg + ash g/kg + crude fiber g/kg)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *HUFA* highly unsaturated fatty acids, *SPC* soybean protein concentrate, *DHC* dihydrocholesterol

consisted of a reference diet (RD) containing 500 g/kg of FM, three diets containing 125 g/kg of experimental meals from shrimp head, Catarina scallop viscera or Pen shell viscera, replacing FM in the RD (diets SD, CD, and PD, respectively), and a diet where the three experimental meals were added at 125 g/kg each, replacing FM (diet SCPD). The formulation of the diets is shown in Table 2.

Table 2 Ingredient composition (g/kg diet) of the experimental and reference diets for longfin almaco jack *Seriola rivoliana* juveniles

Ingredient	SD	CD	PD	SCPD	RD
Fish meal (sardine)[a]	375.0	375.0	375.0	219.8	500.0
Shrimp head meal[b]	125.0			125.0	
Catarina scallop meal[b]		125.0		125.0	
Pen shell meal[b]			125.0	125.0	
Wheat meal[c]	113.8	113.8	113.8	46.5	113.8
Soy protein concentrate[d]	231.7	231.7	231.7	231.1	231.7
Fish oil (cod)[e]	78.6	78.6	78.6	51.8	78.6
Alginate[f]	30.0	30.0	30.0	30.0	30.0
Soy lecithin[g]	20.0	20.0	20.0	20.0	20.0
Vitamin premix[h]	7.0	7.0	7.0	7.0	7.0
Mineral premix[i]	10.0	10.0	10.0	10.0	10.0
Choline chloride[j]	2.0	2.0	2.0	2.0	2.0
Vitamin C[k]	1.0	1.0	1.0	1.0	1.0
Monohydrate betaine[l]	5.0	5.0	5.0	5.0	5.0
β-Carotene[l]	0.8	0.8	0.8	0.8	0.8
BHT[m]	0.1	0.1	0.1	0.1	0.1

Shrimp head diet (SD); Catarina scallop viscera diet (CD); Pen shell viscera diet (PD); and Shrimp head, Catarina and Pen shell viscera (mixed) diet (SCPD); reference diet with FM (RD)

[a]Monterey sardine meal (Conservera San Carlos, Puerto San Carlos, B.C.S., México)

[b]Prepared in our laboratory, CIBNOR

[c]Central de Abastos de La Paz, B.C.S., México

[d]Promotora Industrial Acuasistemas, S.A. de C.V. La Paz, B.C.S., México

[e]Droguería cosmopolita, S.A. de C.V. México, D.F., México

[f]Sigma-Aldrich 180947-05031-1, St. Louis, MO, USA

[g]Rey Sol, La Paz, B.C.S., México

[h]Vitamin premix (mg or IU/kg of diet): vitamin A, 15,000 IU; D_3, 7500 IU; E, 400 mg; K_3, 20 mg; thiamine B_1, 150 mg; riboflavin, 100 mg; pyridoxine B_6, 50 mg; pantothenic acid, 100 mg; niacin, 300 mg; biotin, 1 mg; inositol, 500 mg; folic acid, 20 mg; cyanocobalamin, 0.1 mg

[i]Mineral premix, (g/kg of diet): $MgSO_4$ $7H_2O$, 0.5; $ZnSO_4$ $7H_2O$, 0.09; KCl, 0.5; $MnCl_2$ $4H_2O$, 0.0234; $CuCl_2$ $2H_2O$, 0.005; KI, 0.5; $CoCl_2$ $6H_2O$, 0.0025

[j]62% active agent, ICN Biomedicals Inc., Aurora, OH, USA

[k]Stay-C, 35% active agent. ROCHE, D.F., México

[l]Sigma-Aldrich St. Louis, MO, USA

[m]Butylated hydroxytoluene, ICN Biomedicals Inc., Aurora, OH, USA

The proximate composition, gross energy, total lipids, fatty acids, and sterols content in the diets is shown in Table 3.

Fish and Experimental Design

Seriola rivoliana used for the present study were produced and cultured in our laboratory, as described in Benitez-Hernández et al. [9]. Briefly, ten fish (mean initial weight 48.1 ± 0.6 g) were stocked into each tank. Diets were randomly assigned to triplicate tanks, and fish were manually fed to apparent satiation daily at 08:00, 12:30 and 15:30 h. Fish were individually weighed, and total length was measured on the initial stocking day and once every 15 days until the end of the experiment. Feed intake and fish mortality were recorded daily. Water temperature (29.1 ± 1.0 °C), dissolved oxygen (5.3 ± 1.98 mg/L), and salinity (36.0 ± 5 PSU) were measured daily with a multiparameter (556 MPS, YSI®, YSI Inc., Yellow Springs, OH, USA).

Growth Performance and Feed Intake

Every biometry all fish were caught, anesthetized using a clove oil solution (0.3 mL/L in seawater) and individually weighed and measured. Survival, growth performance and feed intake of the fish was monitored regarding weight gain (WG), specific growth rate (SGR) and feed intake (FI), as follows: Survival (%) = (final number of fish/initial number of fish) × 100; WG (g/org/day) = (final mean weight (g) – initial mean weight (g))/(number of fish)/number of days; SGR (%/day) = 100 [((ln final weight) – (ln initial weight))/time (days)]; FI (g/fish/day) = [(total feed consumption (g))/(number of fish)/number of days].

Fish were sampled from the initial population (n = 5) and from each treatment (n = 6) after a 24-h fast at the end of the experiment. Fish were weighed, measured and 100 mg of each tissue (visceral fat, liver, muscle, and brain) was dissected using a scalpel on a frozen surface and stored separately at − 80 °C for biochemical analyses.

Total Lipids

Total lipids from meals, diets, and fish tissues were analyzed after extraction with chloroform:methanol (2:1 v/v) during 24 h. Total lipids were extracted and analyzed as described by Toyes-Vargas et al. [10]. An aliquot was used for total lipids, which were weighed in an analytical balance (Mettler Toledo, Switzerland) of ± 0.1 mg precision. Other aliquots were used for fatty acids, and sterol analyses, as described below.

Fatty Acids

Aliquots of the lipid extracts were placed in vials containing an internal standard (23:0) and butylated hydroxytoluene (BHT), as described in Palacios et al. [12], using boron-triflouride-methanol (BF3 10% methanol, 3–3021, Sigma-Aldrich, St. Louis, MO) and analyzed in a gas chromatograph 6890 N (Agilent Technologies, Santa Clara, CA) and separated on a DB-23 silica capillary 30 m × 0.25 mm ID × 0.25 um film thickness (50% cyanopropyl-methylpolysiloxane) with helium as carrier gas, a temperature ramp from 110 to 210 °C, and flame

Table 3 Proximate composition (g/kg dry matter), gross energy (MJ/kg), total lipids (g/kg dry matter), fatty acids (g/kg dry matter), and sterols (g/kg dry matter) content in experimental and reference diets

	SD	CD	PD	SCPD	RD
Proximate composition					
Dry matter	944.4	946.9	933.1	907.5	947.2
Crude protein	490.7	493.2	505.4	490.6	488.7
Ether extract	135.8	136.9	129.1	122.5	124.4
Crude fiber	13.2	12.9	23.0	16.9	24.9
Ash	93.1	90.9	102.1	95.4	107.2
NFE[a]	211.6	213.0	173.6	182.2	202.1
Gross energy	20.0	20.2	19.7	19.3	19.7
Total lipids	124.4 ± 1.2	135.8 ± 1.7	136.9 ± 0.3	129.1 ± 0.7	122.4 ± 0.9
Fatty acids					
16:0	9.8 ± 0.2	9.6 ± 0.2	10.5 ± 0.6	8.6 ± 0.5	10.2 ± 0.4
18:0	2.7 ± 0.1	2.5 ± 0.1	2.6 ± 0.2	2.5 ± 0.1	2.5 ± 0.7
16:1n-9	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.01	0.1 ± 0.03	0.2 ± 0.01
16:1n-7	2.8 ± 0.04	2.9 ± 0.1	2.8 ± 0.2	2.4 ± 0.2	2.7 ± 0.2
18:1n-9	13.0 ± 0.1	10.5 ± 0.1	12.1 ± 0.6	7.5 ± 0.5	12.8 ± 0.3
18:1n-7	2.0 ± 0.03	1.7 ± 0.03	2.0 ± 0.1	1.6 ± 0.1	1.9 ± 0.1
18:2n-6	9.3 ± 0.1	7.6 ± 0.2	9.3 ± 0.6	6.2 ± 0.4	9.5 ± 0.6
18:3n-3	1.9 ± 0.6	1.5 ± 0.1	1.9 ± 0.1	1.1 ± 0.1	1.9 ± 0.2
18:4n-3	0.8 ± 0.03	0.7 ± 0.04	1.0 ± 0.1	0.6 ± 0.1	0.9 ± 0.1
18:5n-3	ND	0.01 ± 0.0	ND	0.01 ± 0.0	ND
20:4n-6	0.7 ± 0.02	0.4 ± 0.02	0.5 ± 0.03	0.6 ± 0.1	0.5 ± 0.03
20:5n-3	4.5 ± 0.2	3.3 ± 0.2	5.4 ± 0.4	3.4 ± 0.3	4.3 ± 0.4
22:6n-3	7.9 ± 0.3	6.0 ± 0.4	9.1 ± 0.7	4.9 ± 0.4	9.0 ± 0.8
SFA	15.8 ± 0.3	15.6 ± 0.3	16.4 ± 0.9	14.1 ± 0.8	15.9 ± 0.6
MUFA	24.8 ± 0.5	20.6 ± 0.3	23.2 ± 1.1	15.9 ± 0.8	23.9 ± 0.2
PUFA	28.1 ± 0.7	21.7 ± 1.0	30.1 ± 2.2	18.6 ± 1.5	28.9 ± 2.2
HUFA	16.2 ± 0.6	12.1 ± 0.7	18.3 ± 1.4	10.8 ± 1.0	17.0 ± 1.4
n-3/n-6	1.5 ± 0.03	1.4 ± 0.04	1.7 ± 0.03	1.4 ± 0.02	1.6 ± 0.03
Sterols					
DHC	0.10 ± 0.05	0.09 ± 0.02	0.21 ± 0.03	0.20 ± 0.10	0.10 ± 0.02
Cholesterol	3.3 ± 1.4	2.04 ± 0.6	2.7 ± 0.4	2.2 ± 0.53	2.4 ± 0.4
Brassicasterol	0.17 ± 0.08	0.25 ± 0.08	0.32 ± 0.10	0.38 ± 0.11	0.13 ± 0.01
Campesterol	0.08 ± 0.04	0.15 ± 0.05	0.13 ± 0.04	0.15 ± 0.06	0.08 ± 0.02
Stigmasterol	0.05 ± 0.01	0.17 ± 0.04	0.40 ± 0.10	0.34 ± 0.13	0.05 ± 0.01
β-Sitosterol	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	0.04 ± 0.01
Fucosterol	0.29 ± 0.16	0.38 ± 0.16	0.48 ± 0.12	0.40 ± 0.15	0.29 ± 0.05

Shrimp head diet (SD); Catarina scallop viscera diet (CD); Pen shell viscera diet (PD); Shrimp head, Catarina and Pen shell viscera (mixed) diet (SCPD); reference diet with FM (RD)

Results are expressed as means ± SD, n = 3. *ND* not detected

[a]Nitrogen-free-extract (NFE) = 1000 − (moisture g/kg + crude protein g/kg + ether extract g/kg + ash g/kg + crude fiber g/kg)

ionization detector. The fatty acids were identified by comparing their retention times and external standards (47885-U Supelco, Bellefonte, PA, USA) with ChemStation Rev.A.10.02 (Agilent Technologies) and the concentration of each fatty acid corrected by correlation with the response of the area of the internal standard (T6543, Sigma St. Louis MO, USA).

Sterols

Sterols were analyzed from another aliquot of lipid extract. An internal standard (5-α-cholestane, C8003, Sigma-Aldrich, St. Louis, MO) and BHT were added, and the sample was then transesterified with 2 mL of sodium methoxide-methanol 0.5 N (403067, Sigma) as described previously [13]. The transesterified sample was separated on a silica capillary column (65% difenil-35% dimethylsiloxane, RESTEK, 30 mx 0.25 mm × 0.25 µm) in a gas chromatograph 6890 N Agilent Technologies using hydrogen as carrier with a thermal gradient from 50 to 260 °C, at 5 °C/min, and flame ionization detector, and the peaks were compared to commercial standards (C-8667, C-8003, D-6128, S-2424, E6510, S-1270, Sigma; 03072-5, 06291-10, Alltech, Deerfield, IL, USA).

Biotoxicity Assay (Mouse Bioassay; MBA)

Paralytic Shellfish Toxins (PST) and Diarrhetic Shellfish Toxins (DST)

Identity and quantification of PST (saxitoxin [STX] and analogs). The biological activity was performed by mouse bioassay (MBA) according to AOAC standards (18) [14]. Five g of meal homogenized with 10 ml of 0.1 N HCl, boiled for 5 min, and adjusted to pH 4 with 1 N HCl. The supernatant containing the toxin was obtained by centrifugation at 1100×*g* for 5 min. CD-1 (Harlan Laboratories, Mexico) male mice weighing 18–20 g each, in groups of 3 animals, were injected intraperitoneally with aliquots of 1 mL. The toxicity was determined by the average surviving time in Saxitoxin (STX) FDA Reference Standard (STD). Saxitoxin was obtained from the US National Institute of Standards and Technology (NIST, RM 8642). The saxitoxin STD provided by Marine Toxins and Amino acids Laboratory from CIBNOR. Diarrhetic shellfish toxins (DST) extraction was performed according to the method described by Yasumoto et al. [15, 16] following procedures described by Heredia-Tapia et al. [17] and Campa-Cordova et al. [18]. Twenty-five g of meal was homogenized with 100 mL of 100% acetone. The organic solvent was recovered, and the homogenization step was repeated two times. Acetone extracts were pooled together and roto-evaporated to dryness and the residue was resuspended in 10 mL of saline solution of 1% Tween 60. Aliquots of 500 µL of this extract were injected intraperitoneally into three 18–20 g CD-1 male (Harlan Laboratories, Mexico) strain mice. The concentration of okadaic acid (OA) and dinophysis toxins (DTXs) in the semi-purified extract [19] was calculated as log Mouse Unit (MU) = 2.6 log $(1 + t^{-1})$; MU = 4 µg of OA [20].

Lateral Flow Immunochromatography (LFIC)

Analysis for diarrhetic shellfish toxins (okadaic acid and analogs) were conducted by Lateral flow immunochromatography (LFIC; detection limit 0.08 µg/g OA equiv.) using DSP Scotia Rapid Testing (Scotia Rapid Testing LTD, Nova Scotia, Canada), a qualitative lateral flow screen test for the detection of DST in shellfish.

High Performance Liquid Chromatography Coupled to a Triple Quadrupole Mass Spectrometer (HPLC–MS/MS)

The presence of lipophilic phycotoxins in meal extract was evaluated with liquid chromatography coupled to a triple quadrupole mass spectrometer (HPLC–MS/MS). Toxin extraction and analysis were as described in the European Union Harmonized Standard Operating Procedure for Lipophilic toxins under acidic conditions (EU-SOP-LIP:EURLMB) [21]. Instrument was calibrated with certified reference standards from the National Research Council of Canada (NRC). Toxins analyzed were Domoic acid (DA), Okadaic acid (OA), Dinophysistoxin 1 and 2 (DTX1, DTX2), Pectenotoxins 1 and 2 (PTX2, PTX1), Azaspiracid 1-3 (AZA1-3), Yessotoxin (YTX), homo-yessotoxin (h-YTX), 45-hydroxy yessotoxin (45-OH YTX), 45-hydroxy homo-yessotoxin (45-OH h-YTX), 13-desmethyl spirolide C (13dmSPXC) and Gymnomidine A (GYM). Both unhydrolyzed and hydrolyzed sample extracts were analyzed. Hydrolyzed extracts permitted the quantification OA-group toxins present in acyl-ester form.

Statistical Analysis

Data were tested for normality and homogeneity. Total lipids content was analysed after arc-sin transformation. One-way analysis of variance (ANOVA) was performed with diet as the independent variable. When significant ($p < 0.05$) differences were found, Tukey's test were used for mean comparison [22]. Differences between means in tissues fatty acids and total lipids content at the start and end of the trial were tested using Student's t-test. All statistical analyses were conducted using STATISTICA® 8.0 software package (Stat Soft, Inc., Tulsa, OK, USA).

Results

Ingredients and Diet's Chemical Composition

The ingredients crude protein content ranged between 129 g/kg for wheat meal, to 699.3 g/kg in FM (Table 1). The highest ether extract content was found in Catarina scallop and Pen shell meals (145 and 138 g/kg). Crude fiber content was higher in shrimp head meal (75 g/kg) than in the other meals. The highest ash content (186 g/kg) was found in shrimp head, followed by FM (162 g/kg), and then Catarina scallop meal (86 g/kg). The highest gross energy content (21.5 MJ/kg) was found in Catarina scallop meal and the lowest in shrimp head meal (16.7 MJ/kg). No HUFA content in soybean concentrate or wheat meal was detected. The highest levels of 22:6n-3 and eicosapentaenoic (20:5n-3, EPA) were found in the Pen shell viscera meal, followed by FM and then by shrimp head meal, with lowest levels in Catarina scallop viscera meal. Arachidonic acid (20:4n-6, ARA) was highest in the shrimp head meal, followed by Pen shell viscera meal, then FM, and lastly Catarina scallop viscera meal. A small amount of 18:5n-3 (0.02 mg/kg or 0.06%) was detected in Catarina scallop viscera meal. The most abundant sterol in the animal ingredients was cholesterol, which was higher in shrimp head meal, followed by FM, and then by Catarina scallop viscera meal and Pen shell viscera meal, with no cholesterol in the plant meals. FM also had dihydrocholesterol and brassicasterol, but no other sterol was detected. Shrimp head meal, Catarina scallop viscera meal, Pen shell viscera meal, and plant meals had a wider array of sterols.

The fatty acids, sterols and proximate composition of the diets is reported in Table 3, with 488.7 to 505.4 g/kg of crude protein, 122.5 to 136.9 g/kg of ether extract, 12.9 to 24.9 g/kg of crude fiber, 90.9 to 107.2 g/kg of ash, 182.2 to 213.0 g/kg of nitrogen-free-extract (NFE), and from 19.3 to 20.2 MJ/kg of gross energy. DHA showed higher contents in the Pen shell diet (PD) and RD (9.1 and 9.0 g/kg, respectively), followed by shrimp head diet (SD) and Catarina diet (CD), with lowest levels in the triple diet (SCPD, 4.9 g/kg). EPA content was highest in the PD diet (5.4 g/kg), followed by SD and RD and the CD and SCPD diets had similar concentrations. ARA content was similar in all diets. The fatty acid 18:5n-3 was detected in CD and SCPD diets. This last diet also had the lowest amount of total fatty acids. Cholesterol was highest in the SD, with similar values in the rest of the diets. All phytosterols analyzed were present in all diets.

Growth Performance and Feed Intake

Survival ranged from 90 to 100% and was not significantly affected by treatments. Weight gain, specific growth rate, total length, and feed intake during the 60-day trial are shown in Fig. 1. Weight gain (Fig. 1A) in the first 15 days was higher in fish of the SCPD and PD treatments (4.19 and 4.16 g/org/day) compared to fish of the CD treatment (3.58 g/org/day), but not different from the RD and SD treatments (3.82 and 3.74 g/org/day), and after 30 days fish fed CD and SCPD clearly showed reduced grow (0.36 and 0.85 g/org/day). After 45 days, fish from PD and SD treatments grew faster (5.65 and 5.46 g/org/day) than fish from

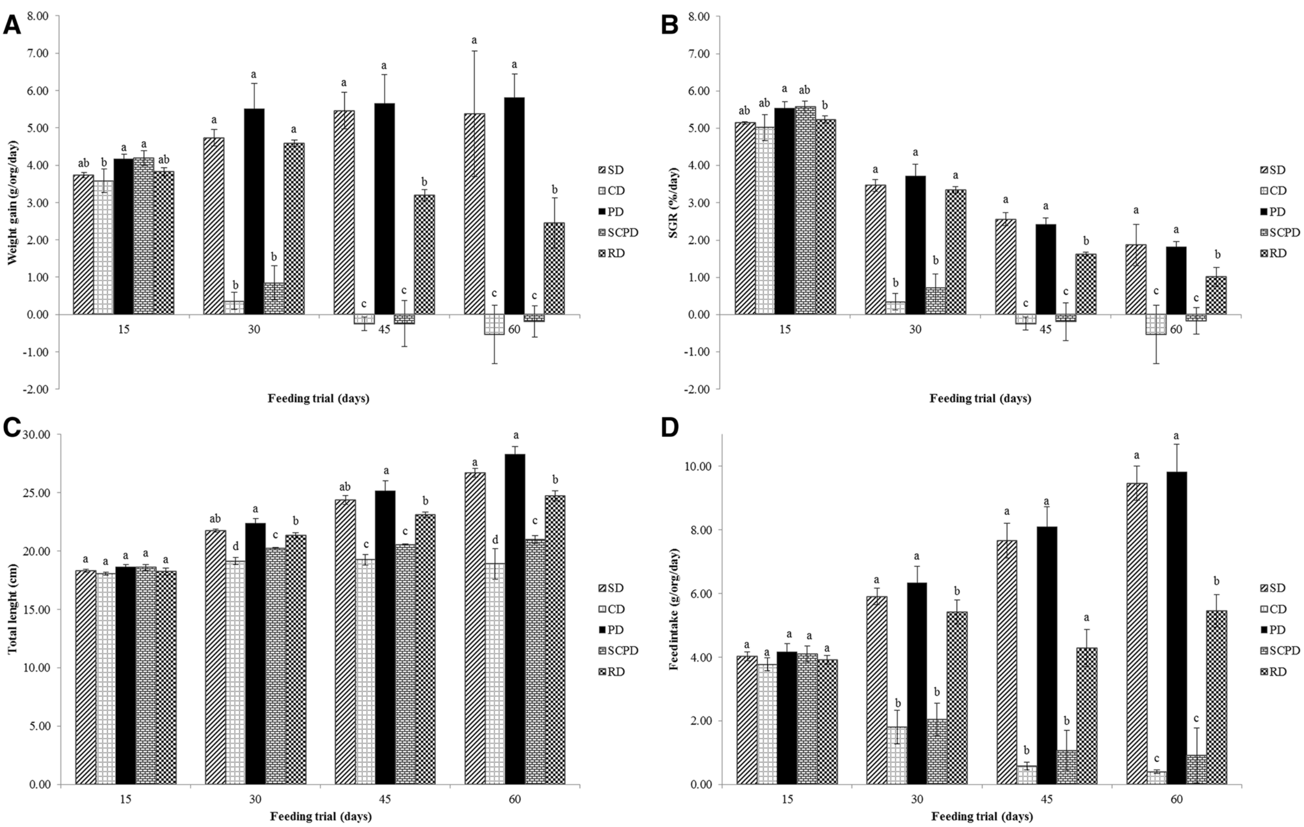

Fig. 1 Weight gain (**A**), specific growth rate (**B**), total length (**C**) and feed intake (**D**) of almaco jack *Seriola rivoliana* juveniles fed the experimental diets containing marine by-product meals. Shrimp head diet (SD); Catarina scallop viscera diet (CD); Pen shell viscera diet (PD); Shrimp head, Catarina, and Pen shell viscera (mixed) diet (SCPD); reference diet (RD). Values with different superscripts within each sampling period are significantly different ($P < 0.05$) according to Tukey's test

RD (3.20 g/org/day), followed by fish from CD and SCPD treatments which exhibited negative growth values (− 0.25 and − 0.24 g/org/day). By the end of the trial, the same pattern was observed with fish from treatments PD and SD attaining weight gain of 5.81 and 5.38 g/org/day while fish from treatments RD grew significantly slower (2.45 g/org/day) as well as fish from treatments SCPD and CD which continued having negative growth (− 0.53 and − 0.19 g/org/day).

Specific growth rate (Fig. 1B) of fish in the PD treatment (5.54%/day) was higher than in fish of the RD at day 15 (5.23%/day), and by days 30 and 45 fish from treatments PD (3.71 and 2.42%/day, respectively) and SD (3.47 and 2.56%/day, respectively) had significantly higher SGR than fish of the RD (3.35 and 1.62%/day, respectively), while fish from treatments CD and SCPD exhibited lower growth from day 30 (0.35 and 0.72%/day), with negative SGR values from day 45 (− 0.24 and − 0.19%/day) and until the end of the trial (− 0.53 and − 0.16%/day).

Total fish length (Fig. 1C) was similar for all treatments at day 15 (18.4 ± 0.2 cm). By day 30, fish from treatment PD (22.4 cm) were significantly larger than fish from the RD (21.4 cm), SCPD (20.3 cm) and CD (19.2 cm) treatments, and similar to fish from treatment SD (21.8 cm). This pattern continued unchanged until the end of the trial, where fish from treatments PD (28.3 cm) and SD (26.7 cm) were significantly larger than fish from the RD (24.7 cm), followed by fish from treatments SCPD (21.0 cm) and CD (18.9 cm) which were smaller than those of the RD treatment.

Feed intake (Fig. 1D) was similar for all treatments at day 15 (4.0 ± 0.2 g/org/day). By day 30, it decreased in fish fed CD and SCPD (1.8 and 2.0 g/org/day), and it decreased even further in these two treatments after 45 (0.58 and 1.06 g/org/day) and 60 days (0.40 and 0.92 g/org/day). In contrast, fish fed PD and SD diets had significantly higher feed intake (9.8 and 9.5 g/org/day) after 60 days in comparison with fish fed RD diet (5.5 g/org/day).

Muscle Lipid Content

The lipid muscle composition is shown in Table 4. Total lipids were highest in muscle from fish fed SD and RD diets (63.0 and 57.0 g/kg, respectively), followed by PD diet and lowest in the muscle of fish fed SCPD and CD diets (19.5 and 15.3 g/kg, respectively). Total lipid content in muscle of fish fed RD, SD and PD diets were similar to that in initial fish (49 g/kg).

Most of the fatty acids in muscle differed in response to diet (Table 4). Saturated fatty acids (SFA) were lowest in the lipids in muscle of fish fed RD, while monounsaturated fatty acids (MUFA) were highest in the same diet and lowest in the lipids of fish fed SCPD. Polyunsaturated fatty acids (PUFA) were highest in the lipids in muscle of fish fed SCPD, with values similar to that of fish at the beginning of the trial, while lowest PUFA levels were found in fish fed RD or SD. HUFA were highest in the lipids of muscle of fish sampled at the beginning of the trial and lowest in fish fed RD and SD, a result of a step decreased in DHA, EPA and ARA in the later.

Cholesterol was the main sterol in muscle, with values ranging between 86 and 93%, with brassicasterol being the second most abundant, with values ranging between 3 and 6%. The highest values for cholesterol were found in fish fed CD, while the lowest were found in muscle of fish fed RD. Brassicasterol levels were not significantly different in muscle as a result of diet, but a minor sterol, fucosterol, was highest in the muscle of fish fed PD.

Total lipids were highest in muscle of fish fed SD and RD, and lowest in fish fed CD and SCPD; three-fold lower than in the fish at the beginning of the trial.

Liver Lipid Content

The lipid composition of liver is shown in Table 5. Total lipids in liver were highest for fish fed PD and RD (214 and 173 g/kg, respectively), with less than half the content in liver from fish fed SD (66.7 g/kg), and half of that in liver of fish fed SCPD or CD (34 and 31 g/kg). Total lipids in liver of fish at the beginning of the trial (109 g/kg) were intermediate between RD and SD.

SFA decreased in liver of fish from all treatments (26–30%) compared to values in liver at the beginning of the trial (32%). This decrease was concomitant with an increase in PUFA from initial values (39%) to 53% in liver of fish fed SCPD. MUFA were highest in lipids of fish fed PD (36%) and lowest in the fish fed SCPD (18%), with the inverse behaviour for HUFA, mainly set by the values of DHA.

Cholesterol ranged from 65% in liver of fish fed CD, to 96% in liver of fish fed SD. In liver, the second more abundant sterol was DHC, with highest values in liver of fish fed PD and RD (29 and 22%, respectively), and lower values in fish fed CD (3.5%), similar to that of fish at the beginning of the trial (9%). No DHC was detected in the liver of fish fed SD of SCPD.

Brain Lipid Content

The lipid composition in brain of almaco jack is shown in Table 6. Total lipid content in brain was highest in the fish fed RD (138 g/kg), followed by SD and PD (102 and 85 g/kg, respectively), and lowest in the CD and SCPD (49 and 59 g/kg, respectively). These last had levels similar to brains in initial fish (49 g/kg).

SFA were highest at the beginning of the experiment (37%) and decreased in all treatments, with the lowest values found in lipids in brain of fish fed PD (29%), while MUFA

Table 4 Total lipids (g/kg as is), fatty acids (% of total fatty acids) and sterols (% of total sterols) in the muscle of *Seriola rivoliana* fed the experimental diets

	Initial	SD	CD	PD	SCPD	RD
Total lipids	49.0 ± 6.2ab	63.0 ± 28.0a	15.3 ± 2.4c	31.1 ± 0.7b	19.5 ± 8.0c	57.0 ± 10.6a
Fatty acids						
14:0	1.6 ± 0.3ab	2.2 ± 0.3b	1.1 ± 0.4ab	2.1 ± 0.2b	0.8 ± 0.2a	2.3 ± 0.1b
16:0	18.6 ± 0.3a	16.0 ± 0.3c	17.7 ± 0.2ab	17.4 ± 0.1abc	16.8 ± 0.5bc	16.0 ± 0.4c
18:0	7.0 ± 0.1ab	5.5 ± 0.7c	7.9 ± 0.4ab	6.5 ± 0.1bc	8.8 ± 0.3a	5.1 ± 0.4c
20:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:0	ND	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.0	ND
24:0	0.2 ± 0.0	0.2 ± 0.1	0.5 ± 0.2	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
SFA	28.8 ± 0.6a	25.6 ± 0.8bc	28.9 ± 0.5a	28.0 ± 0.2ab	28.1 ± 0.5ab	24.9 ± 0.7c
15:1n-8	0.2 ± 0.0	ND	0.5 ± 0.2	ND	0.5 ± 0.1	ND
16:1n-9	0.2 ± 0.0	0.2 ± 0.0a	0.8 ± 0.3b	0.3 ± 0.0a	0.8 ± 0.1b	0.3 ± 0.0a
16:1n-7	3.5 ± 0.4a	3.5 ± 0.5	1.8 ± 0.5	3.2 ± 0.2	1.4 ± 0.3	3.6 ± 0.3
18:1n-9	9.9 ± 0.6a	18.9 ± 1.7c	12.0 ± 1.5ab	16.6 ± 1.0bc	10.1 ± 1.4a	19.3 ± 1.4c
18:1n-7	2.8 ± 0.1ab	3.0 ± 0.1b	2.3 ± 0.1a	2.9 ± 0.1	2.4 ± 0.1a	2.9 ± 0.1b
20:1n-11	ND	0.6 ± 0.1	ND	ND	ND	ND
20:1n-9	0.9 ± 0.1a	2.6 ± 0.2b	1.4 ± 0.3a	2.5 ± 0.1b	1.1 ± 0.2a	2.8 ± 0.1b
20:1n-7	ND	0.3 ± 0.0	ND	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:1n-11	0.4 ± 0.1	1.9 ± 0.3	ND	ND	ND	ND
22:1n-9	ND	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	ND	2.2 ± 0.1
24:1n-9	1.7 ± 0.2	1.0 ± 0.3	3.5 ± 1.2	0.9 ± 0.2	2.9 ± 0.5	1.1 ± 0.3
MUFA	20.4 ± 1.0a	33.1 ± 2.6b	23.0 ± 1.9a	27.7 ± 1.2ab	19.9 ± 2.1a	33.0 ± 1.7b
18:2n-6	5.0 ± 0.2a	12.2 ± 0.9c	9.6 ± 0.6bc	11.3 ± 0.4bc	8.7 ± 0.9b	12.8 ± 0.8c
18:3n-6	0.3 ± 0.0a	0.2 ± 0.0b	0.2 ± 0.0b	0.2 ± 0.0b	0.2 ± 0.0b	0.2 ± 0.0b
18:3n-3	0.7 ± 0.1a	2.0 ± 0.3b	0.8 ± 0.2a	1.8 ± 0.1b	0.8 ± 0.2a	2.2 ± 0.2b
18:4n-3	ND	ND	0.2 ± 0.1	0.2 ± 0.0	ND	0.2 ± 0.0
18:5n-3	ND	ND	ND	ND	ND	ND
20:2n-6	0.3 ± 0.0a	0.6 ± 0.0c	0.4 ± 0.0b	0.5 ± 0.0c	0.4 ± 0.0b	0.6 ± 0.0c
20:3n-3	ND	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:4n-6	2.6 ± 0.2c	1.3 ± 0.3ab	2.5 ± 0.4bc	1.0 ± 0.1a	3.9 ± 0.4d	0.8 ± 0.1a
20:5n-3	10.8 ± 0.2a	4.5 ± 0.2c	5.4 ± 0.2bc	5.5 ± 0.1bc	6.2 ± 0.4b	4.7 ± 0.0c
21:4n-6	ND	0.8 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	ND	0.8 ± 0.1
22:4n-6	0.9 ± 0.1a	0.4 ± 0.1b	0.8 ± 0.0a	0.4 ± 0.0b	1.0 ± 0.1a	0.4 ± 0.0b
22:5n-6	ND	0.5 ± 0.2	0.9 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
22:5n-3	3.9 ± 0.1a	2.8 ± 0.1b	2.4 ± 0.2b	2.9 ± 0.1b	2.8 ± 0.1b	2.8 ± 0.0b
22:6n-3	25.9 ± 1.4ab	15.3 ± 3.0b	24.1 ± 2.7ab	19.1 ± 1.6ab	27.1 ± 3.0a	15.9 ± 1.8b
PUFA	50.8 ± 1.6a	41.3 ± 2.0b	48.2 ± 2.1ab	44.3 ± 1.2ab	52.0 ± 2.0a	42.1 ± 1.0b
HUFA	44.5 ± 1.9a	25.7 ± 3.2c	36.6 ± 2.8abc	30.0 ± 1.7bc	41.6 ± 3.0ab	25.9 ± 2.0c
n-3/n-6	4.5 ± 0.2 a	1.6 ± 0.2b	2.3 ± 0.3b	2.0 ± 0.1b	2.5 ± 0.3b	1.6 ± 0.2b
Sterols						
DHC	0.4 ± 0.0	1.3 ± 0.6	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.9 ± 0.2
Cholesterol	90.6 ± 0.8ab	90.7 ± 0.9ab	92.5 ± 0.7b	90.3 ± 0.5ab	91.3 ± 1.3ab	86.8 ± 1.9a
Brassicasterol	4.9 ± 0.4	3.4 ± 0.1	4.0 ± 0.2	4.2 ± 0.2	4.8 ± 0.6	6.1 ± 2.5
Campesterol	1.0 ± 0.3	0.4 ± 0.1	0.3 ± 0.0	0.8 ± 0.1	0.3 ± 0.1	0.6 ± 0.3
Stigmasterol	1.6 ± 0.1a	2.4 ± 0.2ab	1.5 ± 0.2a	1.8 ± 0.1ab	1.6 ± 0.2a	2.8 ± 0.4b
β-Sitosterol	0.7 ± 0.1a	1.1 ± 0.2ab	0.7 ± 0.1a	0.7 ± 0.1a	0.7 ± 0.1a	1.4 ± 0.1b
Fucosterol	0.8 ± 0.2ab	0.7 ± 0.0b	0.7 ± 0.1b	1.6 ± 0.2a	0.9 ± 0.4ab	1.3 ± 0.5ab

Results are expressed as means ± SE, n = 3. Means with different superscripts within the same row are significantly different ($P < 0.05$) according to Tukey's test. Means without superscripts are not significantly different. See Table 3 for other abbreviations

Table 5 Total lipids (g/kg as is), fatty acids (% of total fatty acids) and sterols (% of total sterols) in the liver of *Seriola rivoliana* fed the experimental diets

	Initial	SD	CD	PD	SCPD	RD
Total lipids	108.6 ± 27.0ab	66.7 ± 19.8b	33.1 ± 5.1c	214.0 ± 49.6a	34.0 ± 5.5c	173.0 ± 15.9a
Fatty acids						
14:0	2.4 ± 0.1a	1.5 ± 0.2ab	1.3 ± 0.5ab	1.7 ± 0.3ab	0.8 ± 0.3b	1.6 ± 0.4ab
16:0	21.2 ± 0.3a	16.3 ± 0.7c	17.8 ± 0.3bc	17.7 ± 0.3bc	18.3 ± 0.4b	16.9 ± 0.2bc
18:0	6.8 ± 0.5	6.1 ± 0.4	6.1 ± 0.6	7.1 ± 0.5	8.7 ± 0.8	6.1 ± 0.5
20:0	ND	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	ND
22:0	ND	ND	ND	ND	0.2 ± 0.1	ND
24:0	ND	ND	ND	ND	ND	ND
SFA	32.4 ± 0.8a	25.5 ± 1.2c	26.8 ± 0.4bc	28.0 ± 0.3bc	29.6 ± 0.9ab	26.0 ± 0.4c
15:1n-8	ND	ND	ND	ND	ND	ND
16:1n-9	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.1
16:1n-7	5.7 ± 0.2a	2.7 ± 0.4b	2.2 ± 0.9b	3.0 ± 0.4b	1.3 ± 0.4b	2.6 ± 0.5b
18:1n-9	14.5 ± 1.0ab	17.8 ± 3.0ab	12.4 ± 3.8a	21.2 ± 0.7ab	8.0 ± 1.2b	18.7 ± 4.1ab
18:1n-7	4.2 ± 0.3a	3.0 ± 0.2ab	2.6 ± 0.4ab	3.6 ± 0.1ab	2.5 ± 0.3b	3.0 ± 0.3ab
20:1n-11	0.3 ± 0.0	ND	ND	1.0 ± 0.1	0.3 ± 0.2	ND
20:1n-9	0.8 ± 0.1a	2.1 ± 0.2abc	1.3 ± 0.3abc	2.5 ± 0.1c	1.0 ± 0.3ab	2.4 ± 0.6bc
20:1n-7	0.2 ± 0.0	0.3 ± 0.0	ND	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
22:1n-11	0.4 ± 0.0	1.3 ± 0.2	1.2 ± 0.3	2.0 ± 0.1	1.1 ± 0.2	1.8 ± 0.6
22:1n-9	ND	0.2 ± 0.0	ND	0.2 ± 0.0	ND	0.2 ± 0.1
24:1n-9	1.4 ± 0.3	1.5 ± 0.4	1.9 ± 0.6	1.1 ± 0.3	2.7 ± 0.6	1.1 ± 0.3
MUFA	28.5 ± 1.3ab	30.1 ± 3.8ab	23.0 ± 5.3ab	35.7 ± 0.6a	17.8 ± 2.1b	31.0 ± 5.9ab
18:2n-6	7.3 ± 0.1	11.9 ± 0.9	10.1 ± 1.8	12.0 ± 0.5	7.5 ± 0.8	12.4 ± 1.5
18:3n-6	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
18:3n-3	0.8 ± 0.0ab	1.8 ± 0.2a	1.0 ± 0.3ab	1.8 ± 0.3a	0.6 ± 0.2b	1.7 ± 0.3a
18:4n-3	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.2 ± 0.1	0.5 ± 0.1
18:5n-3	ND	ND	ND	ND	ND	ND
20:2n-6	0.5 ± 0.0	0.8 ± 0.0	0.5 ± 0.3	0.7 ± 0.1	0.6 ± 0.0	0.8 ± 0.1
20:3n-3	ND	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:4n-6	2.6 ± 0.2ab	2.8 ± 0.7ab	3.8 ± 1.2ab	1.3 ± 0.2b	5.6 ± 0.7a	1.9 ± 0.7b
20:5n-3	7.7 ± 0.6a	5.2 ± 0.5ab	5.6 ± 0.4ab	4.8 ± 0.4b	5.8 ± 0.6ab	4.8 ± 0.9b
21:4n-6	1.0 ± 0.0a	0.7 ± 0.1ab	0.5 ± 0.1b	0.8 ± 0.0ab	0.4 ± 0.0b	0.8 ± 0.1ab
22:4n-6	0.5 ± 0.0ab	0.3 ± 0.1ab	0.6 ± 0.1bc	0.2 ± 0.0a	0.7 ± 0.1c	0.3 ± 0.1ab
22:5n-6	ND	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1
22:5n-3	3.7 ± 0.0a	2.3 ± 0.1b	2.4 ± 0.2b	2.1 ± 0.1b	2.7 ± 0.3b	2.5 ± 0.1b
22:6n-3	13.8 ± 1.6bc	17.3 ± 4.3bc	24.6 ± 5.4ab	11.1 ± 1.1c	27.6 ± 2.1a	16.4 ± 5.1bc
PUFA	39.1 ± 2.1c	44.4 ± 4.7bc	50.2 ± 5.0ab	36.3 ± 0.6c	52.5 ± 1.4a	43.0 ± 4.9bc
HUFA	29.9 ± 2.2bc	29.4 ± 5.5bc	38.1 ± 6.9ab	21.1 ± 0.9c	43.2 ± 2.4a	27.4 ± 6.7bc
n-3/n-6	2.2 ± 0.2ab	1.6 ± 0.3ab	2.1 ± 0.4ab	1.3 ± 0.0b	2.4 ± 0.2a	1.6 ± 0.5ab
Sterols						
DHC	9.1 ± 2.6a	ND	3.5 ± 1.4a	28.7 ± 4.1b	ND	22.2 ± 1.7b
Cholesterol	83.7 ± 3.1b	95.9 ± 0.4a	92.2 ± 1.5ab	64.9 ± 3.2c	94.0 ± 0.3a	73.7 ± 1.8c
Brassicasterol	2.3 ± 0.3a	1.8 ± 0.2ab	1.6 ± 0.1ab	0.6 ± 0.3b	2.3 ± 0.6a	0.6 ± 0.2b
Campesterol	1.7 ± 0.3a	0.4 ± 0.1bc	1.0 ± 0.1ab	0.6 ± 0.1bc	1.7 ± 0.2a	0.3 ± 0.1c
Stigmasterol	2.6 ± 0.5a	1.3 ± 0.2a	1.2 ± 0.3a	4.8 ± 0.8b	1.5 ± 0.2a	3.0 ± 0.1ab
β-Sitosterol	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	0.2 ± 0.0
Fucosterol	ND	ND	ND	ND	ND	ND

Results are expressed as means ± SE, n = 3. See Table 3 for abbreviations and Table 4 for statistical analyses

Table 6 Total lipids (g/kg as is), fatty acids (% of total fatty acids) and sterols (% of total sterols) in the brain of *Seriola rivoliana* fed the experimental diets

	Initial	SD	CD	PD	SCPD	RD
Total lipids	48.7 ± 6.3c	101.9 ± 15.6b	48.8 ± 4.2c	84.6 ± 13.5b	59.0 ± 9.6c	138.4 ± 3.8a
Fatty acids						
14:0	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	1.7 ± 0.6	0.6 ± 0.3	0.9 ± 0.2
16:0	15.8 ± 0.5	14.6 ± 1.3	12.7 ± 0.3	14.7 ± 0.4	13.5 ± 0.3	14.4 ± 0.9
18:0	12.9 ± 0.2	12.4 ± 1.1	11.8 ± 0.8	8.4 ± 1.4	12.4 ± 0.8	11.8 ± 1.3
20:0	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1
22:0	1.0 ± 0.1	1.0 ± 0.2	1.5 ± 0.1	0.9 ± 0.2	1.4 ± 0.0	1.1 ± 0.3
24:0	6.0 ± 0.4	5.0 ± 1.4	4.7 ± 1.1	2.5 ± 0.8	5.2 ± 0.2	3.9 ± 1.1
SFA	37.3 ± 0.1a	34.8 ± 1.2ab	32.4 ± 1.3bc	29.3 ± 1.3c	34.1 ± 0.4ab	32.9 ± 1.2bc
15:1n-8	1.6 ± 0.1b	1.6 ± 0.2b	2.6 ± 0.1a	1.4 ± 0.4b	2.7 ± 0.1a	1.7 ± 0.3b
16:1n-9	2.0 ± 0.1ab	2.1 ± 0.2ab	3.2 ± 0.1a	1.9 ± 0.4b	3.2 ± 0.1a	2.2 ± 0.4ab
16:1n-7	1.9 ± 0.3	1.9 ± 0.2	1.8 ± 0.4	3.1 ± 0.7	1.6 ± 0.4	2.0 ± 0.3
18:1n-9	14.8 ± 0.6a	19.3 ± 2.0b	20.7 ± 0.8b	20.3 ± 0.5b	19.3 ± 0.6b	20.2 ± 1.9b
18:1n-7	1.8 ± 0.1	2.0 ± 0.0	1.8 ± 0.2	2.3 ± 0.3	1.8 ± 0.2	1.9 ± 0.1
20:1n-11	ND	ND	ND	ND	ND	ND
20:1n-9	0.4 ± 0.1	1.0 ± 0.1	0.6 ± 0.2	1.5 ± 0.4	0.6 ± 0.3	1.1 ± 0.2
20:1n-7	ND	0.2 ± 0.0	ND	0.2 ± 0.0	ND	0.2 ± 0.0
22:1n-11	0.2 ± 0.0a	0.7 ± 0.1ab	0.4 ± 0.2ab	1.2 ± 0.3b	0.3 ± 0.1ab	0.8 ± 0.2ab
22:1n-9	ND	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
24:1n-9	1.3 ± 0.1a	1.9 ± 0.4ab	2.7 ± 0.1b	1.6 ± 0.3ab	2.3 ± 0.2ab	2.1 ± 0.3ab
MUFA	24.5 ± 0.7a	31.3 ± 3.2b	34.4 ± 1.4b	34.2 ± 1.2b	32.6 ± 1.4b	32.6 ± 3.3b
18:2n-6	1.5 ± 0.3a	4.1 ± 0.5ab	2.8 ± 0.9ab	7.5 ± 2.1b	2.3 ± 1.0ab	4.4 ± 1.0ab
18:3n-6	ND	ND	ND	ND	ND	ND
18:3n-3	0.3 ± 0.1a	0.7 ± 0.1ab	0.3 ± 0.2a	1.4 ± 0.5b	0.3 ± 0.2a	0.7 ± 0.2ab
18:4n-3	ND	ND	ND	ND	ND	ND
18:5n-3	ND	ND	ND	ND	ND	ND
20:2n-6	ND	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
20:3n-3	ND	ND	ND	ND	ND	ND
20:4n-6	2.0 ± 0.1ab	1.8 ± 0.1ab	1.7 ± 0.1bc	1.4 ± 0.2c	2.2 ± 0.0a	1.5 ± 0.2bc
20:5n-3	3.8 ± 0.3ab	2.7 ± 0.2b	2.9 ± 0.3ab	4.7 ± 0.7a	2.6 ± 0.4b	3.0 ± 0.3ab
21:4n-6	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	0.4 ± 0.1
22:4n-6	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
22:5n-6	ND	ND	ND	ND	ND	ND
22:5n-3	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.1
22:6n-3	29.3 ± 0.5a	23.0 ± 3.0ab	23.8 ± 1.6ab	19.5 ± 3.3b	24.3 ± 2.9ab	23.1 ± 3.3ab
PUFA	38.1 ± 0.7	33.9 ± 2.6	33.2 ± 0.3	36.5 ± 0.4	33.3 ± 1.2	34.4 ± 2.9
HUFA	36.0 ± 0.5	28.6 ± 2.9	29.6 ± 1.3	26.9 ± 2.6	30.3 ± 2.5	28.8 ± 3.2
n-3/n-6	7.6 ± 0.6a	3.9 ± 0.6ab	5.1 ± 1.0ab	2.8 ± 0.7b	5.3 ± 1.2ab	4.1 ± 1.0ab
Sterols						
DHC	ND	1.8 ± 0.4	2.3 ± 0.1	1.9 ± 0.6	2.2 ± 0.2	2.1 ± 0.3
Cholesterol	94.6 ± 0.4	96.0 ± 0.1	95.7 ± 0.2	94.9 ± 0.4	94.6 ± 0.3	95.8 ± 0.1
Brassicasterol	2.3 ± 0.1a	1.1 ± 0.2bc	0.5 ± 0.1c	1.5 ± 0.2ab	1.5 ± 0.3ab	0.7 ± 0.2bc
Campesterol	1.4 ± 0.2a	ND	0.3 ± 0.0b	0.1 ± 0.0b	0.1 ± 0.0b	0.1 ± 0.0b
Stigmasterol	0.3 ± 0.1bc	0.1 ± 0.0c	0.4 ± 0.0ab	0.5 ± 0.1ab	0.6 ± 0.1a	0.3 ± 0.1bc
β-Sitosterol	0.8 ± 0.1a	0.7 ± 0.1ab	0.5 ± 0.1b	0.7 ± 0.0ab	0.7 ± 0.0ab	0.6 ± 0.1ab
Fucosterol	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.1

Results are expressed as means ± SE, n = 3. See Table 3 for abbreviations and Table 4 for statistical analyses

increased in all treatments (31–34%) compared to values in lipids in brain of initial fish (25%). PUFA and HUFA were not significantly different among treatments, although DHA was highest at the beginning (29%) and lowest in the fish fed PD (20%).

Mesenteric Fat Composition

Fish at the beginning of the trial had 423 g/kg total lipids in mesenteric fat. After the trial, total lipids were highest in fat of fish fed SD (681 g/kg), followed by PD (588 g/kg), RD (546 g/kg), CD (472 g/kg), and lowest in SCPD (251 g/kg, Table 7).

SFA were reduced from 33% in fat of fish at the beginning of the trial, to 25% in fish fed RD and SD, while MUFA were increased in all treatments (34–38%) compared to initial values (29%). HUFA decreased from 28% in the initial animals, to 16–21% in the treatments.

Cholesterol levels were around 71–76% for all treatments, without a significant treatment.

Biotoxicity Assay (Mouse Bioassay; MBA)

Paralytic Shellfish Toxins (PST) and Diarrhetic Shellfish Toxins (DST)

Negative activity of paralytic shellfish toxins (PST) by mouse bioassay (MBA) in all the experimental meals was found; the only clinical signs observed when Catarina scallop meal was tested were lethargy and in one out of three mice respiratory failure in the first 15 min, without any other signs. The clinical signs presented by MBA exposed to diarrhetic shellfish toxins were hind limb paralysis, spasms, respiratory failure, immobility, lethargy, and locomotion problems. In two out of three mice severe diarrhea, and in one case dyspnoea and death within 24 h. These signs are similar to those described for diarrhetic shellfish toxins (OA and analogues) [23].

Lateral Flow Immunochromathography (LFIC)

Positive identification of DST by qualitative analysis (okadaic acid and analogs) for Lateral flow immunochromathography (LFIC) was found in Catarina scallop meal (Fig. 2).

High Performance Liquid Chromatography Coupled to a Triple Quadrupole Mass Spectrometer (HPLC–MS/MS)

The presence of okadaic acid (OA) and dinophysistoxin 1 (DTX1) was confirmed by the HPLC–MS/MS method. Of the different phycotoxins evaluated, only AO and DTX-1were positively identified and quantified in the Catarina scallop meal (Fig. 3). OA and DTX1 specific fragments were detected after the disruption of their parent ion 803.2 *m/z* and 817.5 *m/z*, respectively (Fig. 3 B, C). The calculated concentration of OA was 27.64 µg/g, and 10.31 µg/g for DTX1.

Discussion

Given the nature of the experimental ingredients and the research that has already been done previously in shrimp [5–7, 10, 24] we were rather confident that the by-products tested here would meet the profile to partially replace FM in the feed for carnivorous fish. It resulted true in the case of shrimp head (SD) and Pen shell viscera meals (PD). However, almaco jack juveniles fed CD and SCPD had negative specific growth rate at the end of the trial (− 0.53 and − 0.16%/day), much lower that the RD (1.01%/day) or the other two experimental diets, SD (1.87%/day) and PD (1.82%/day). Previous analyses of other batches of CD showed low concentrations of essential fatty acids, which are necessary for a rapid growth of *S. rivoliana.* Catarina scallop meal did have much less HUFA than any of the other by-products, with values of HUFA around 1.2 g/kg dw, while shrimp head meal had 11.2, Pen shell meal had 29.6 and FM had 17.3 g/kg dw. The requirements of HUFA n-3 for *Seriola* species, range between 5.0 and 20.0 g/kg of EPA + DHA in the diet [25, 26]. Here we found EPA + DHA in the feed ranged from 12.4 to 14.5 g/kg of the diet for RD, SD, and PD, but were below 10 g/kg for CD and SCPD, but even so these diets had sufficient HUFA n-3 according to literature, so HUFA "low" levels was probably not to blame.

We supposed that the triple diet would be the best for growth since nutrients that were lacking in one by-product could be provided by another by-product. For example, scallops in general are low in cholesterol and rich in phytosterols not present in FM. In contrast, shrimp contains three-fold the cholesterol of scallops (Table 1). However, the growth results of fish fed the SCPD were similar to that of the CD, both much lower than the other treatments (RD, SD, and PD). These very low growth led us to suppose that Catarina scallop viscera meal was contaminated: Here, we found that Catarina scallop viscera meal did have low but nonetheless detectable levels of 18:5n-3 (0.02 g/kg). This fatty acid is found in marine dinoflagellates, such as *Gymnodinium* sp. or *Prorocentrum* sp. [27]. The presence of this fatty acid could indicate that Catarina scallops were in contact with dinoflagellates, probably from an initiating red tide, that they were ingested and that the toxins were accumulated in the viscera of the Catarina scallops. In situ, when the viscera of the Catarina scallops were collected, we noticed no evidence of contamination. However, a slightly higher mortality than usual for the season was reported, attributed to the higher water temperature during the summer (fishermen of the

Table 7 Total lipids (g/kg as is), fatty acids (% of total fatty acids) and sterols (% of total sterols) in the mesenteric fat of *Seriola rivoliana* fed the experimental diets

	Initial	SD	CD	PD	SCPD	RD
Total lipids	423.3 ± 17.2c	681.3 ± 7.0a	471.5 ± 37.9c	588.2 ± 38.5b	250.5 ± 6.3d	545.6 ± 19.9b
Fatty acids						
14:0	4.6 ± 0.1ab	3.4 ± 0.1bc	4.9 ± 0.2a	3.0 ± 0.3c	4.5 ± 0.5ab	3.4 ± 0.1bc
16:0	18.5 ± 0.4a	14.1 ± 0.5b	15.2 ± 0.0b	15.8 ± 0.5b	14.9 ± 0.6b	14.6 ± 0.3b
18:0	4.9 ± 0.3	3.6 ± 0.2	5.0 ± 0.2	4.9 ± 0.8	5.0 ± 0.8	3.6 ± 0.0
20:0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
22:0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
24:0	3.1 ± 0.1	2.7 ± 0.0	2.9 ± 0.1	2.9 ± 0.5	3.1 ± 0.2	2.8 ± 0.1
SFA	32.9 ± 0.5a	25.4 ± 0.6b	29.8 ± 0.4ab	28.2 ± 0.9ab	29.4 ± 2.3ab	25.8 ± 0.5b
15:1n-8	ND	ND	ND	0.3 ± 0.3	ND	ND
16:1n-9	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.4 ± 0.0
16:1n-7	8.6 ± 0.3a	5.3 ± 0.1b	5.4 ± 0.5b	4.8 ± 0.4b	5.1 ± 0.1b	5.2 ± 0.2b
18:1n-9	13.9 ± 0.2a	21.1 ± 0.5b	21.2 ± 0.3b	20.2 ± 0.9b	21.2 ± 0.1b	19.9 ± 0.2b
18:1n-7	3.3 ± 0.0	3.1 ± 0.0	3.1 ± 0.0	3.1 ± 0.1	3.3 ± 0.1	2.9 ± 0.0
20:1n-11	ND	ND	ND	ND	ND	ND
20:1n-9	0.8 ± 0.0a	2.6 ± 0.1bc	3.4 ± 0.4c	2.3 ± 0.1b	3.1 ± 0.2bc	2.4 ± 0.1b
20:1n-7	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.2 ± 0.0
22:1n-11	0.4 ± 0.0a	2.1 ± 0.1bc	2.9 ± 0.5c	1.7 ± 0.1b	2.4 ± 0.1bc	1.9 ± 0.1bc
22:1n-9	ND	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
24:1n-9	0.3 ± 0.0a	0.5 ± 0.0ab	0.8 ± 0.1b	0.6 ± 0.2ab	0.6 ± 0.0ab	0.6 ± 0.0ab
MUFA	28.6 ± 0.4a	36.5 ± 0.7b	38.4 ± 0.9c	34.3 ± 0.8b	37.8 ± 0.4c	34.4 ± 0.4b
18:2n-6	7.8 ± 0.1a	15.3 ± 0.2b	12.9 ± 0.1b	13.2 ± 0.9b	13.7 ± 1.0b	14.5 ± 0.2b
18:3n-6	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
18:3n-3	1.4 ± 0.0	3.1 ± 0.2	1.8 ± 0.1	2.7 ± 0.2	2.0 ± 0.4	3.0 ± 0.1
18:4n-3	0.2 ± 0.0	0.2 ± 0.0	ND	0.2 ± 0.0	ND	0.2 ± 0.0
18:5n-3	ND	ND	ND	ND	ND	ND
20:2n-6	0.3 ± 0.0a	0.6 ± 0.0b	0.6 ± 0.0b	0.5 ± 0.0b	0.6 ± 0.0b	0.6 ± 0.0b
20:3n-3	ND	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:4n-6	2.0 ± 0.1a	1.0 ± 0.1b	0.9 ± 0.0bc	1.0 ± 0.1b	1.3 ± 0.1b	0.8 ± 0.1c
20:5n-3	12.5 ± 0.5a	6.3 ± 0.5b	4.4 ± 0.6b	6.8 ± 0.8b	4.6 ± 0.6b	6.8 ± 0.1b
21:4n-6	1.0 ± 0.0	1.0 ± 0.0	0.8 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.1 ± 0.0
22:4n-6	0.5 ± 0.0a	0.3 ± 0.0b	0.3 ± 0.0b	0.3 ± 0.0b	0.4 ± 0.0ab	0.3 ± 0.0b
22:5n-6	ND	ND	ND	ND	ND	ND
22:5n-3	ND	ND	ND	ND	ND	ND
22:6n-3	12.0 ± 0.5a	9.4 ± 0.4bc	9.1 ± 0.5c	11.6 ± 0.7ab	8.5 ± 0.5c	11.6 ± 0.4ab
PUFA	38.5 ± 0.1ab	38.1 ± 1.3ab	31.8 ± 1.3b	38.0 ± 1.2ab	32.8 ± 2.7ab	39.8 ± 0.6a
HUFA	28.3 ± 0.1a	18.5 ± 0.9bc	15.9 ± 1.3c	21.0 ± 1.0b	15.9 ± 1.2c	21.1 ± 0.5b
n-3/n-6	2.2 ± 0.0a	1.0 ± 0.0c	1.0 ± 0.1c	1.4 ± 0.1b	0.9 ± 0.0c	1.2 ± 0.0bc
Sterols						
DHC	18.9 ± 1.5	15.2 ± 3.4	19.7 ± 1.7	20.8 ± 1.8	16.3 ± 0.0	15.8 ± 1.9
Cholesterol	75.4 ± 1.3	75.7 ± 3.9	72.6 ± 2.5	70.8 ± 1.8	76.3 ± 0.0	75.6 ± 4.0
Brassicasterol	ND	ND	ND	ND	ND	ND
Campesterol	ND	ND	ND	ND	ND	ND
Stigmasterol	5.7 ± 0.6	8.1 ± 1.1	7.7 ± 0.8	7.2 ± 0.3	5.7 ± 0.0	8.6 ± 2.2
β-Sitosterol	ND	ND	ND	ND	ND	ND
Fucosterol	ND	ND	ND	ND	ND	ND

Results are expressed as means ± SE, n = 3. See Table 3 for abbreviations and Table 4 for statistical analyses

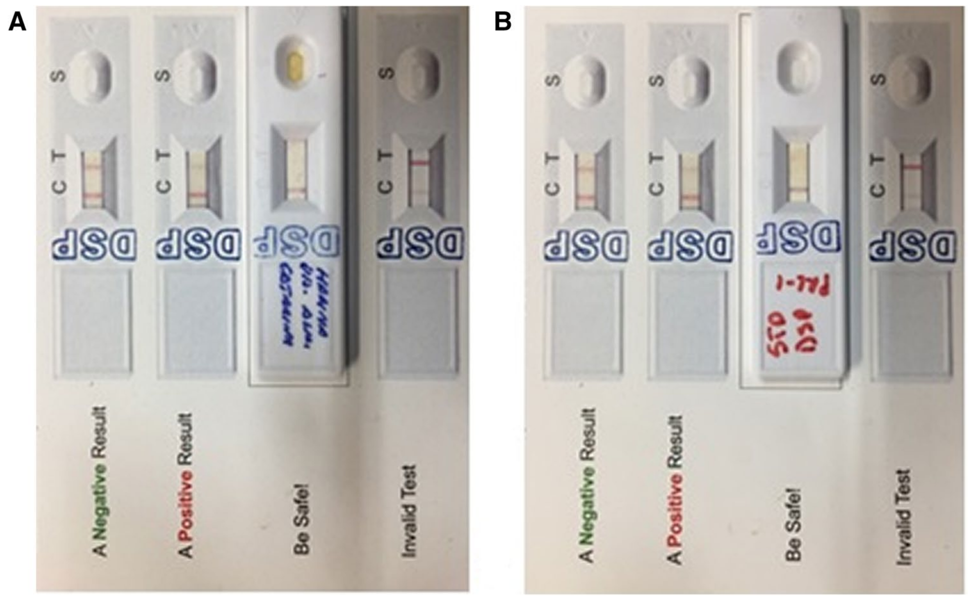

Fig. 2 Positive identification of DST toxins by analysis for lateral flow immunochromatography (LFIC; detection limit 0.08 μg/g okadaic acid equiv.). **A** Viscera meal-extract of the Catarina scallop; **B** Control positive (DST Internal standard)

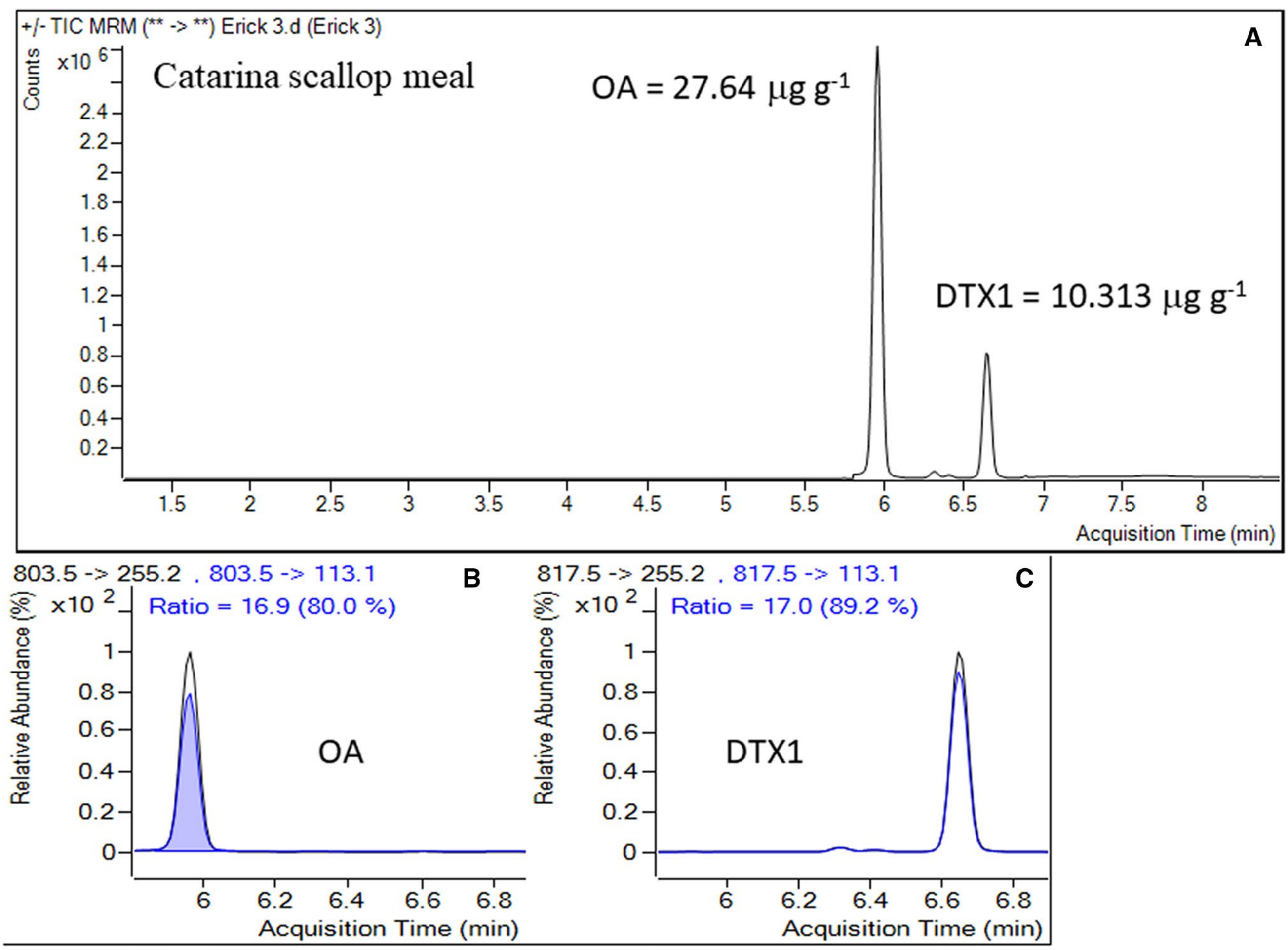

Fig. 3 Total Ion Counts (TICs) obtained following HPLC–MS/MS analysis of Catarina scallop meal (**A**). TICs of the 800.3 255.2 *m/z*, 800.3 113.1 *m/z* transitions (**B**) are characteristic of the fragmentation of the OA molecule. The 817.5 255.2 *m/z*, 817.3 113.1 *m/z* transitions are associated to Dinophysis toxin 1 (DTX1; **C**)

community 2014, *pers. comm.*). Dinoflagellates (*Dinophysis* spp. and *Prorocentrum* spp.) that produce diarrhetic toxins (OA group) have been registered in the Gulf of California [16, 28, 29] in Todos Santos Bay, Northern Pacific coast of Mexico [30], in the Magdalena-Almejas lagoon system, B.C.S, from 1980 to 1989 and during 2005 and 2006 [31], and in Laguna Ojo de Liebre, B.C.S., from May to June 2014 [28, 32]. Some species of *Dinophysis* and *Prorocentrum* produce toxins, particularly OA and DTXs that are accumulated by bivalve mollusks, such as oysters and clams after consuming these dinoflagellates [33, 34].

The harmful effect of OA and its analogues under controlled conditions in the reproduction, on the early stages of development of aquatic organisms [35] and marine fish has been previously described [36–39]. DST administrated via diet or dissolved in seawater affect marine fish at different life stages (from embryo to adults) [39] of *S. rivoliana* [40, 41]. It is difficult to establish a dose threshold that could affect fish species since there is a limited number of studies related to the dietary exposure of these organisms to DST. These toxins have been administrated through *Artemia* exposed to toxin-producing dinoflagellates [36] and there is only one study in which the effect of DST toxins in artificial feed was evaluated [38, 39]. The exposure to 1300 µg/kg OA eq. in feed affected the swimming performance of the Zebra seabream (*Diplodus cervinus*) [38]. Juveniles of the almaco jack were exposed to approximately 24 times this concentration (37,955 µg /kg OA eq. assuming DTX1 has the same toxicity as OA: toxicity factor of 1). Therefore, a clear negatively affect was evident when the almaco jack juveniles were chronically exposed to this toxin concentration in feed. Dissolved DST significantly inhibit protein and alkaline phosphatases, affecting the regulation pathways associated with embryogenesis, altering gene expression, and affecting the viability and lipid metabolism in *S. rivoliana* embryo [40, 41]. Similar alterations could be associated with the long-term ingestion of DST present in artificial diets.

When diets (CD and SCPD) containing Catarina scallop viscera meal were offered to fish, they initially ate them at the same rate as the fish in other treatments, indicating that palatability was not affected, and fish initially probably did not detect an off-flavor in the feed. However, after some days, fish were observed to actively reject the CD and SCPD feeds, by nipping the pellets as they sank in the water column and then spitting them out. This is consistent with a learned discomfort, probably in the digestive tract. The feeding intake was similar at the beginning of the experiment (3.8 and 4.1 g/day for each fish, for CD and SCPD, respectively), with a slight decrease at 15 days, but by day 30 it had reduced to half, 1.8 and 2.0 g/org/day in fish fed CD and SCPD, and it decreased even further after 45 and 60 days, while in the others treatments, feeding intake was of 5.5 g/org/day for the RD, 9.5 g/org/day for SD and 9.8 g/org/day for PD at the end of the trial. Decreased feeding in CD and SCPD is in accordance with a lack of growth in these two treatments. By the end of the trial weight decreased to − 0.53 and − 0.19%/day in CD and SCPD fed juveniles. In agreement, total lipids in muscle and liver of fish fed CD and SCPD were significantly lower than fish sampled at the beginning of the experiment, indicating that the fat fish started with had been exhausted, instead of accumulated, as was the case in the other treatments. Interestingly, total lipids in brain did not decrease in CD and SCPD fed fish and remained fairly similar to initial levels, denoting a differential use of fat from different tissues.

The effect of the long-held non-intentional fasting in the juveniles fed CD and SCPD on the fatty acid accumulation are also interesting. These diets had similar concentration of total lipids and ARA compared to RF, although lower levels of DHA in both diets (Table 3). However, the proportion of DHA in lipids in muscle were significantly higher in fish fed SCPD compared to the RD; it should be noted that fish fed this diet had very low concentration of total lipids (Table 4), thus, the absolute levels of DHA are lower in muscle of fish fed SCPD (~5 g/kg) compared to fish fed RD (~9 g/kg). Nevertheless, this difference in DHA absolute levels is little less than a two-fold decrease compared to the control, while total lipids decreased almost three-fold, indicating a selective conservation of DHA in muscle during the imposed fast for fish fed CD and SCPD. Clearly, juveniles struggled to maintain some essential fatty acids necessary for survival, which most likely accumulated in the phospholipid fraction in detriment of triacylglicerides, as DHA present in phospholipids is essential for neural tissue, sensory organs, and skeletal system [42]. However, DHA concentration in mesenteric fat in juveniles fed CD and SCPD was similar to other treatments, indicating that even with this level of fasting, fat was not burned to cover for essential fatty acid necessities in other tissues. These would indicate a much more regulated lipid metabolism in mesenteric fat that previously though, and not just a deposit of excess fatty acids from feed [43, 44]. DHA in brain had similar concentrations and proportions in all treatments. Interestingly, juveniles fed PD had much more DHA accumulated in the muscle compared to liver, in comparison to all other treatments, indicating that there might be other component in PD that help the transference of DHA from liver to other tissues. The concentration of ARA was stable in all tissues despite differences in treatments, indicating a stronger conservation of this fatty acid compared to others during the forced fasting, even more so than DHA. ARA is the substrate of eicosanoids that are needed for immune response, maturation, growth, etc., so its levels are tightly regulated in cells [45].

Putting aside the effect of a possible contamination with dinoflagellates containing toxins of the Catarina scallop viscera meal, substituting FM with shrimp head meal or Pen shell viscera meal gave very good results in juvenile *S. rivoliana*. Pen shell viscera meal had even higher levels of EPA (12.6 g/kg) and DHA (12.1 g/kg) than FM (Table 1) with much lower DHA levels in shrimp head meal (3.4 g/kg) compared to FM (11.5 g/kg), but similar values of EPA between shrimp head meal and FM (4.0 and 3.4 g/kg). ARA levels were also higher in Pen shell viscera meal and shrimp head meal compared to FM. These differences in the meals were reflected in the diets (Table 3): PD with slightly higher levels of DHA and EPA compared to SD and RD. The n-3/n-6 ratio was similar among the feeds, but the DHA/EPA ratio was higher for RD, even if the PD had more DHA, since it also had more EPA. Several studies have suggested that DHA/EPA ratio in diet is important for marine fish [46, 47]. In studies using feed with different ratios of DHA/EPA ranging from 0.8 to 1.7, the best results on growth of *Seriola* sp. [28, 48] were obtained using the highest ratio (DHA/EPA 1.5 to 1.7). Here, the three diets were equal or above this ratio (1.7–2.1), and we did obtain very good daily weight gain in all three diets (Fig. 1 A). In tissues, the DHA/EPA increased compared to initial values and to diets; in muscle values ranged from 3.4 for RD to 3.5 in the PD (without considering the diets containing Catarina meal), indicating a stronger accumulation of DHA relative to EPA in the last. In liver, the ratio increased from 1.8 in the initial fish to 2.6 in the RD, and to 3.2 in SD. In mesenteric fat, from 0.97 to 1.7 in the RD. The only exception was the brain, where the initial values were 7.9, and they significantly decreased to 5.7 in the PD, mostly given by a greater increase of EPA in brain tissue of PD fed juveniles, with no significant differences with the other two treatments. PD fed juveniles also had the highest increase of EPA and DHA in mesenteric fat, suggesting an accumulation of these HUFA from diet. In contrast, levels of these two fatty acids in muscle of juveniles fed PD were lower compared to SD and RD, suggesting a differential transference and accumulation depending on the source of fatty acids. This could be a result of where these fatty acids are stored, i.e., acylglycerides or phospholipids, or if they are attached to different kinds of phospholipids. In contrast to FM, marine by-products are rich in lipid reserves that are composed of triacylglycerides [10]. HUFA can be digested, absorbed, and accumulated differently when united to an acylglyceride, such as in fish oil, or to a phospholipid [49]. In this case, Pen shell viscera meal was obtained from viscera of Pen shell that had a developed gonad, which has a very high proportion of vitellin that is composed of phospholipids.

One interesting difference between SD and the other diets was the very high levels of cholesterol in the former. Most fish can synthetize cholesterol, so it is generally not actively included in the feed. We expected more cholesterol accumulation in the tissues of juveniles fed SD, but particularly in liver, levels were lower compared to the initial values or to the RD. The liver uses cholesterol to produce bile so it aids in digestion [50, 51], and an excess of cholesterol in the diet might reduce the need to accumulate cholesterol in this tissue. It is possible that the higher concentration of cholesterol in the diet stimulates the synthesis of bile salts in juveniles almaco jack. In liver, cholesterol is also used to produce lipoproteins and aid lipid transport in the blood [52], in accordance with a slight, also not significant, increase of total lipids in muscle of juveniles fed SD.

In all, it is concluded that the inclusion of some marine by-product meals, in this case, shrimp heads and Pen shell viscera, can reduce the use of FM in the diet, allowing to maintain or even improve the fatty acid profile (HUFA) and the cholesterol content in the different tissues of *S. rivoliana* compared to the RD diet. From a human nutritional point of view, the almaco jack fillets (muscle) had levels of DHA and HUFA similar to RD with FM, when fed SD. However, as an experience derived from the present study, it is important to perform a prior toxicity analysis to rule out any type of toxin in the marine by-products, particularly those that are prone to filtrate and accumulate lipidic toxins, as is the case of bivalve mollusks.

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Data Availability Data are available under request.

Code Availability Not applicable.

Declarations

Conflict of interest The authors confirm no conflict of interest.

Ethical Approval This work adheres to CICUAL-CIBNOR, the institution's care and usage committee obtained prior to the start of the study. We followed the guidelines specified in NOM-033-SAG/ZOO-2014 for animal welfare https://www.dof.gob.mx/nota_detalle.php?codigo=5405210&fecha=26/08/2015 specifically for anesthesia using clover oil before euthanasia, we used those specified by Jenkins et al. (2014) "Guidelines for the Use of Fishes in Research". Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland. We followed the guidelines specified in mouse model "NOM-062-ZOO-1999 Technical specifications for production, care and use of laboratory animals" https://www.fmvz.unam.mx/fmvz/principal/archivos/062ZOO.PDF and Hedrich et al. (2004). The Laboratory Mouse. The Handbook of Experimental Animals. Elsevier Academic Press.

Consent to Participate and Publication All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content.

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