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 produce from fisheries; most of these by-products are discarded. However, these by-products are rich in highly unsaturated fatty acids that are not synthetized by most marine animals that are produced by aquaculture. Here, we used three marine by-products (shrimp head, Catarina scallop viscera, and Pen shell viscera) to produce meals without the step used to separate lipids as is traditionally done; this separation can promote hydrolysis of lipids and ultimately, oxidation of fatty acids and sterols. Lipid-rich meals were used to partially substitute commercial fishmeal 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 had a better effect on growth, lipid, and phytosterols levels. However, Catarina meal had lower concentration of 20:4n-6 and 22:6n-3 but 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 these scallops, albeit their healthy aspect, were in contact with toxins (okadaic acid) produced by dinoflagellates, a setback that needs to be addressed previous to meal manufacturing. In conclusion, marine by-products processed to maintain lipid composition can be used to reduce the use of fishmeal in the diet, and their use improve the lipid content and growth compared to control diet with fishmeal.

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 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.

1. 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 highly unsaturated fatty acids (HUFA), which have to 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, 8, 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 antinutrients, 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 *Seriola rivoliana* juveniles, or be actively rejected, affecting their growth and hematological parameters [9]. Carnivorous fish with high growth rates like the almaco jack *Seriola rivoliana* requires 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 have antinutrients that affect fish but not shrimp. Finally, the feeds have to not only promote growth, but 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 *Seriola 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 fishmeal in feeds.

2. 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 minutes. 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.

Five diets containing ~500 g/kg crude protein and ~130 g/kg ether extract were prepared as described by Civera and Guillaume [11] and evaluated in a 60-day growth trial reported in our previous study [9].

The dietary treatments consisted of a reference diet (RD) containing 500 g/kg of FM, 113.8 g/kg of wheat meal, 231.7 g/kg of soy protein concentrate, 78.6 g/kg of fish oil, 30.0 g/kg of sodium alginate, and 20 g/kg of soy lecithin. Also, three diets containing experimental meals from shrimp head, Catarina scallop viscera or Pen shell viscera, were added at 125 g/kg, replacing FM in the reference diet (diets SD, CD, and PD, respectively), and a diet where the three experimental meals were added at 125 g/kg, replacing FM (diet SCPD). All diets contained 25.9 g/kg of a mixture of the micro-ingredients (vitamin and mineral premixes, dibasic sodium phosphate, choline chloride, vitamin C and BHT as antioxidant) at constant dietary levels as described in Benitez-Hernández et al. [9].

Fish and experimental design

The *S. rivoliana* juveniles used for the present study were obtained from the 60-day feeding trial previously conducted in our laboratory, described in Benitez-Hernández et al. [9](2018). 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 on the initial stocking day and 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 (36,000±500 mg/L) were measured daily with a multiparameter (556 MPS, YSI®, YSI Inc., Yellow Springs, OH, USA).

Sample collection and chemical analysis

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 tissues were dissected using a scalpel on a frozen surface. Each tissue (visceral fat, liver, muscle, and brain) was weighed separately, and stored 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](2016). 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 separated in a gas chromatograph (6890N, Agilent Technologies, Santa Clara, CA) with DB-23 silica capillary column (50% cyanopropyl-methylpolysiloxane) (30 m × 0.25 mm ID × 0.25 µm film thickness) with helium as carrier gas, a temperature ramp from 110 to 210°C, and flame ionization detector. The identification fatty acid was further verified by comparison of their retention times with standards using 23:0 (T6543, Sigma, St. Louis, MO) as internal standard, and a commercial mixture (47885-U, Supelco, Bellafonte, PA) as external standards.

Sterols

Sterols were analyzed from another aliquot of lipid extract to which an internal standard (5-α-cholestane) and BHT were added, were transesterified with 2 mL of sodium methoxide-methanol 0.5N (403067, Sigma) as described previously [13](Palacios, Racotta, Arjona, Marty, Le Coz, Moal, & Samain, 2007b) and separated on a silica capillary column (65% difenil-35% dimethylsiloxane, RESTEK, 30 mx 0.25 mm x 0.25 µm) using hydrogen as carrier with a thermal gradient from 50 to 260°C, at 5°C/min, and flame ionization detector. The internal standard was cholestane (C8003, Sigma-Aldrich, St. Louis, MO) and commercial standards (C-8667, C-8003, D-6128, S-2424, E6510, S-1270, Sigma; 03072-5, 06291-10, Alltech, Deerfield, IL, USA) were used as external standards.

Total carotenoids

Aliquots from the lipid extracts were evaporated to dryness under nitrogen and re-suspended in acetone; absorbance was read at 470, 653 and 666 nm using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Total carotenoids concentrations were calculated as described in Tolasa, Cakli, and Ostermeyer [14].

Statistical analysis

All data were tested for normality and homogeneity. Total lipids content was analysed after arc-sin transformation. One-way analysis of variance (ANOVA) were performed with diet as the independent variable. When significant (p<0.05) differences were found, a Tukey's test was used for mean comparison [15]. 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).

This work adheres to CICUAL-CIBNOR, the institution's care and usage committee obtained prior to the start of the study.

3. Results

Ingredients and experimental diets composition

The composition of the ingredients in shown in Table 1. No HUFA content in soybean concentrate or wheat meal was detected. The highest level 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 proximate composition of the diets has been reported before, and ranged from 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, and from 19.3 to 20.2 MJ/kg of gross energy [9].

The fatty acids and sterol content of the diets are shown in Table 2. DHA showed higher contents in the Pen shell diet (PD) and reference diet (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 diet and in SCPD diet. 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. Carotenoid levels were highest in the PD, and lowest in the CD and SCPD.

Muscle lipid content

The lipid muscle composition is shown in Table 3. 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 3). SFA were lowest in the fish fed RD, while monounsaturated fatty acids (MUFA) were highest in the same diet and lowest in the fish fed SCPD. Polyunsaturated fatty acids (PUFA) were highest in the muscle of fish fed SCPD, with values similar to that of fish at the beginning of the assay, while lowest PUFA levels were found in fish fed RD or SD. HUFA were highest in muscle of fish sampled at the beginning of the assay 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 assay.

Liver lipid content

The lipid composition of liver is shown in Table 4. 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 (173 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 bioassay (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 assay (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 fish fed PD (36%) and lowest in the fish fed SCPD (18%), with the inverse behavior 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 bioassay (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 5. 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 brain of fish fed PD (29%), while MUFA increased in all treatments (31-34%) compared to values 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 bioassay had 423 g/kg total lipids in mesenteric fat. After the bioassay, 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 6).

SFA were reduced from 33% in fat of fish at the beginning of the assay, 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.

4. Discussion

Given the nature of the experimental ingredients and the research that has already been done previously in shrimp [5-7, 10, 16] 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, from the growth results reported before [9] we knew that almaco jack fed CD and SCPD had very low specific growth rate, 1.1 and 1.5% a day, respectively, half that of the RD (2.8 %) or the other two experimental diets, SD (3.2 %) and PD (3.4 %). 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 g/kg dw, 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 5.0-20.0 g/kg of EPA + DHA in the diet [17, 18]. 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, so even these diets had sufficient HUFA n-3 according to literature. We assumed 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 (117.1 g) were similar to that of the CD (95.2 g), lower than the other treatments: RD (258.9 g), SD (328.9 g) and PD (365.1 g) [9]. We then supposed that Catarina scallop viscera meal could have been 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, 18:5n-3 is found in marine dinoflagellates, such as *Gymnodinium sp.* or *Prorecentrum sp*. [19]. The presence of this fatty acid could indicate that Catarina scallops were in contact with dinoflagellates, probably in the form of 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 community 2014, pers. comm.). There have been reports of the presence of dinoflagellates (*Gambierdiscus toxicus, Prorocentrum mexicanum* and *P. rhathymum*) producers of diarrheic toxins such as okadaic acid in the Magdalena-Almejas lagoon system, B.C.S, Mexico in the periods 1980-1989 and 2005-2006 [20] and again in the Laguna Ojo de Liebre, B.C. S., from May to June 2014 [21, 22]. These types of dinoflagellates produce toxins, particularly okadaic acid that are accumulated by bivalve mollusks, such as oysters and clams in their tissues after consuming these dinoflagellates [23]. After detecting 18:5n-3, we tested all meals for toxins using immunochromatography test of lateral flow and found that the Catarina meal used here did contain low levels (0.02 g/kg) of

okadaic acid (Nuñez-Vázquez 2018, pers. comm.). 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, 1.6 and 2.0 g/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 5.4 g/day for the RD, 5.9 g/day for SD and 6.3 g/day for PD for each organism. 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 10.9% and 5.2 % in CD and SCPD fed juveniles, compared to their weights at day 30 [9]. 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 levels of lipid, ARA and DHA compared to the RD. However, the concentration of DHA (Fig. 1) and ARA (Fig. 2) differed among diets. The concentration of DHA was significantly lower in muscle of the CD and SCPD fed juveniles compared the initial values, RD and SD, even if the proportion of DHA in muscle was significantly higher, particularly for the SCPD fed juveniles, indicating a selective conservation of DHA in muscle for CD and SCPD during the imposed fast. Clearly, juveniles struggled to maintain some essential fatty acids necessary for survival, and DHA is essential for neural tissue, sensory organs and skeletal system [24]. 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 lipid necessities. 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 [25, 26]. DHA in brain had similar concentrations and proportions of DHA 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 from liver to other tissues. The concentration of ARA (Fig. 2) was fairly 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 [27].

Putting aside the effect of a possible contamination with dinoflagellates 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 in particular, 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 2): 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 the particular diet DHA/EPA ratio is important for marine fish [28, 29]. 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. [18, 30] 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 (2.8 % for RD, 3.2 % for SD, and 3.4 % for PD) [9]. 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 even 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 [31].

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. Another explanation, is that gonad of Pen shell accumulates great amounts of carotenoids (Table 2) that can reduce the oxidation of lipids during the meal-production process [10]. Phospholipids in particular, can be very prone to peroxidation [32], and since Pen shell viscera meal had the highest carotenoid content, a higher quality of lipids in this meal can be expected.

One difference between SD and the other diets was the very high levels of cholesterol in the former. Most fish are able to 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 [33, 34], 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 [35], in accordance to 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 fishmeal 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 assay, 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 mollusks.

Declarations

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The authors confirm no conflict of interest.

Ethics approval (include appropriate approvals or waivers)

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.

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.

Availability of data and material (data transparency):

Data are available under request

Code availability (software application or custom code): not applicable

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Tables

Table 1 Fatty acids (g/kg dry matter) and sterols (g/kg dry matter) content in the ingredients used for the experimental diets

	Fishmeal	SPC	Wheat meal	Shrimp head meal	Catarina viscera meal	Pen shell viscera meal
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	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	ND	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. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; HUFA: Highly unsaturated fatty acids. SPC= Soybean protein concentrate. DHC= Dihydrocholesterol.

Table 2 Total lipids (g/kg dry matter), fatty acids (g/kg dry matter), sterols (g/kg dry matter), and carotenoid (mg/kg dry matter) content in experimental diets

	RD	SD	CD	PD	SCPD
Total Lipids	122.4±0.9	124.4±1.2	135.8±1.7	136.9±0.3	129.1±0.7
Fatty Acids					
16:0	10.2±0.4	9.8±0.2	9.6±0.2	10.5±0.6	8.6± 0.5
18:0	2.5±0.7	2.7±0.1	2.5±0.1	2.6±0.2	2.5±0.1
16:1n-9	0.2±0.01	0.3±0.0	0.1±0.0	0.1±0.01	0.1±0.03
16:1n-7	2.7±0.2	2.8±0.04	2.9±0.1	2.8±0.2	2.4±0.2
18:1n-9	12.8±0.3	13.0±0.1	10.5±0.1	12.1±0.6	7.5±0.5
18:1n-7	1.9±0.1	2.0±0.03	1.7±0.03	2.0±0.1	1.6±0.1
18:2n-6	9.5±0.6	9.3±0.1	7.6±0.2	9.3±0.6	6.2±0.4
18:3n-3	1.9±0.2	1.9±0.6	1.5±0.1	1.9±0.1	1.1±0.1
18:4n-3	0.9±0.1	0.8±0.03	0.7±0.04	1.0±0.1	0.6±0.1
18:5n-3	ND	ND	0.01±0.0	ND	0.01±0.0
20:4n-6	0.5±0.03	0.7±0.02	0.4±0.02	0.5±0.03	0.6±0.1
20:5n-3	4.3±0.4	4.5±0.2	3.3±0.2	5.4±0.4	3.4±0.3
22:6n-3	9.0±0.8	7.9±0.3	6.0±0.4	9.1±0.7	4.9±0.4
SFA	15.9±0.6	15.8±0.3	15.6±0.3	16.4±0.9	14.1±0.8
MUFA	23.9±0.2	24.8±0.5	20.6±0.3	23.2±1.1	15.9±0.8
PUFA	28.9±2.2	28.1±0.7	21.7±1.0	30.1±2.2	18.6±1.5
HUFA	17.0±1.4	16.2±0.6	12.1±0.7	18.3±1.4	10.8±1.0
n-3/n-6	1.6±0.03	1.5±0.03	1.4±0.04	1.7±0.03	1.4±0.02
Sterols					
DHC	0.10±0.02	0.10±0.05	0.09±0.02	0.21±0.03	0.20±0.10
Cholesterol	2.4±0.4	3.3±1.4	2.04±0.6	2.7±0.4	2.2±0.53
Brassicasterol	0.13±0.01	0.17±0.08	0.25±0.08	0.32±0.10	0.38±0.11
Campesterol	0.08±0.02	0.08±0.04	0.15±0.05	0.13±0.04	0.15±0.06
Stigmasterol	0.05±0.01	0.05±0.01	0.17±0.04	0.40±0.10	0.34±0.13
β-Sitosterol	0.04±0.01	0.03±0.01	0.02±0.01	0.05±0.01	0.01±0.00
Fucosterol	0.29±0.05	0.29±0.16	0.38±0.16	0.48±0.12	0.40±0.15
Total Carotenoids	80.4±6.7	71.4±1.4	41.4±3.8	97.3±3.8	37.6±3.7

Results are expressed as means ± SD, n=3. Reference diet with FM (RD); 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). ND = not detected. See Table 1 for other abbreviations.

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

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 2 for other abbreviations.

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

Results are expressed as means ± SE, n=3. See Table 3 for statistical analyses and Table 2 for abbreviations.

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

Results are expressed as means ± SE, n=3. See Table 3 for statistical analyses and Table 2 for abbreviations.

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

Results are expressed as means ± SE, n=3. See Table 3 for statistical analyses and Table 2 for abbreviations.

Figures

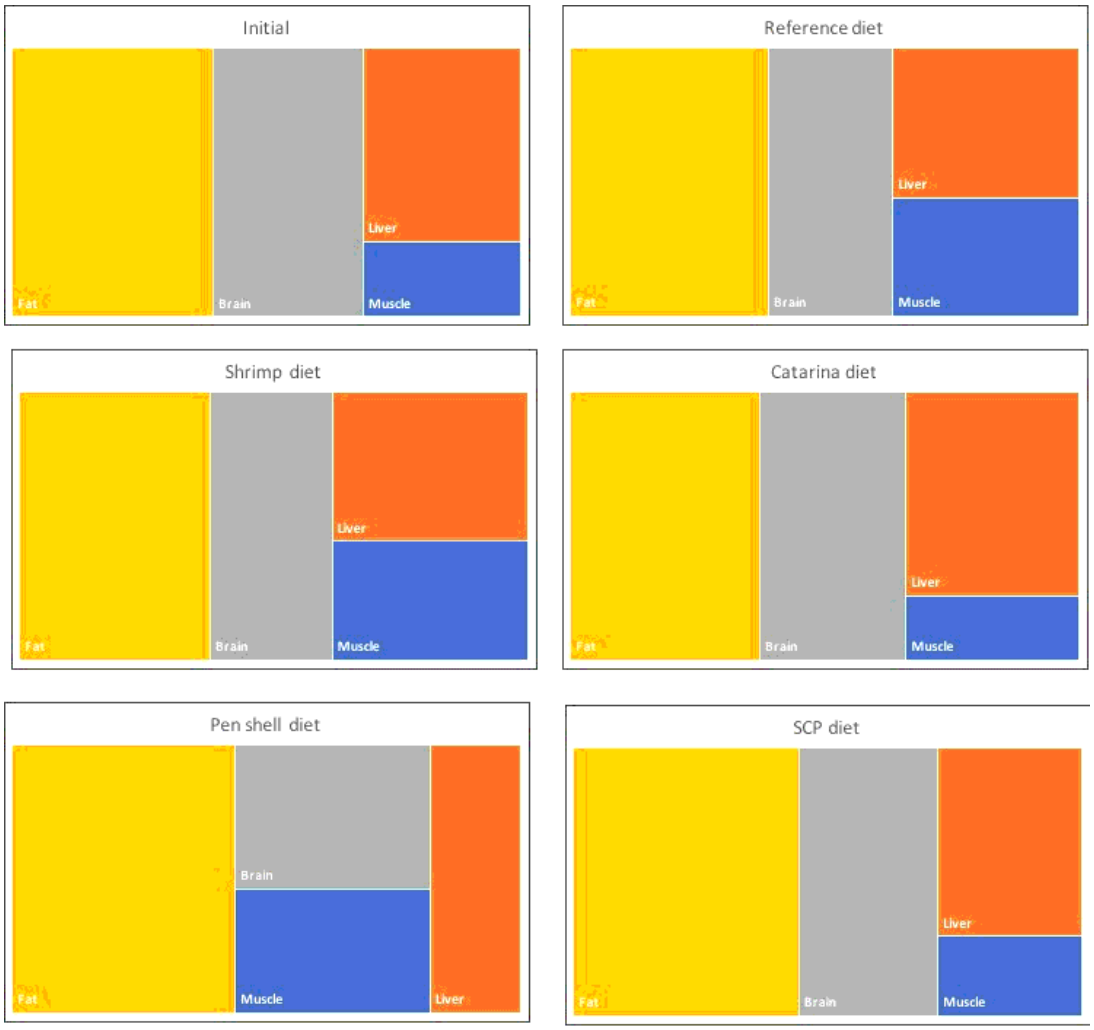

Figure 1

Concentration of 22:6n-3 (DHA, mg/g dw) content in muscle, liver, brain and mesenteric fat of S. rivoliana at the beginning of the bioassay (Initial) or after 60-d of feeding on a Reference diet; Shrimp head diet; Catarina scallop viscera diet; Pen shell viscera diet; and Shrimp head, Catarina and Pen shell viscera (mixed) diet (SCPD).

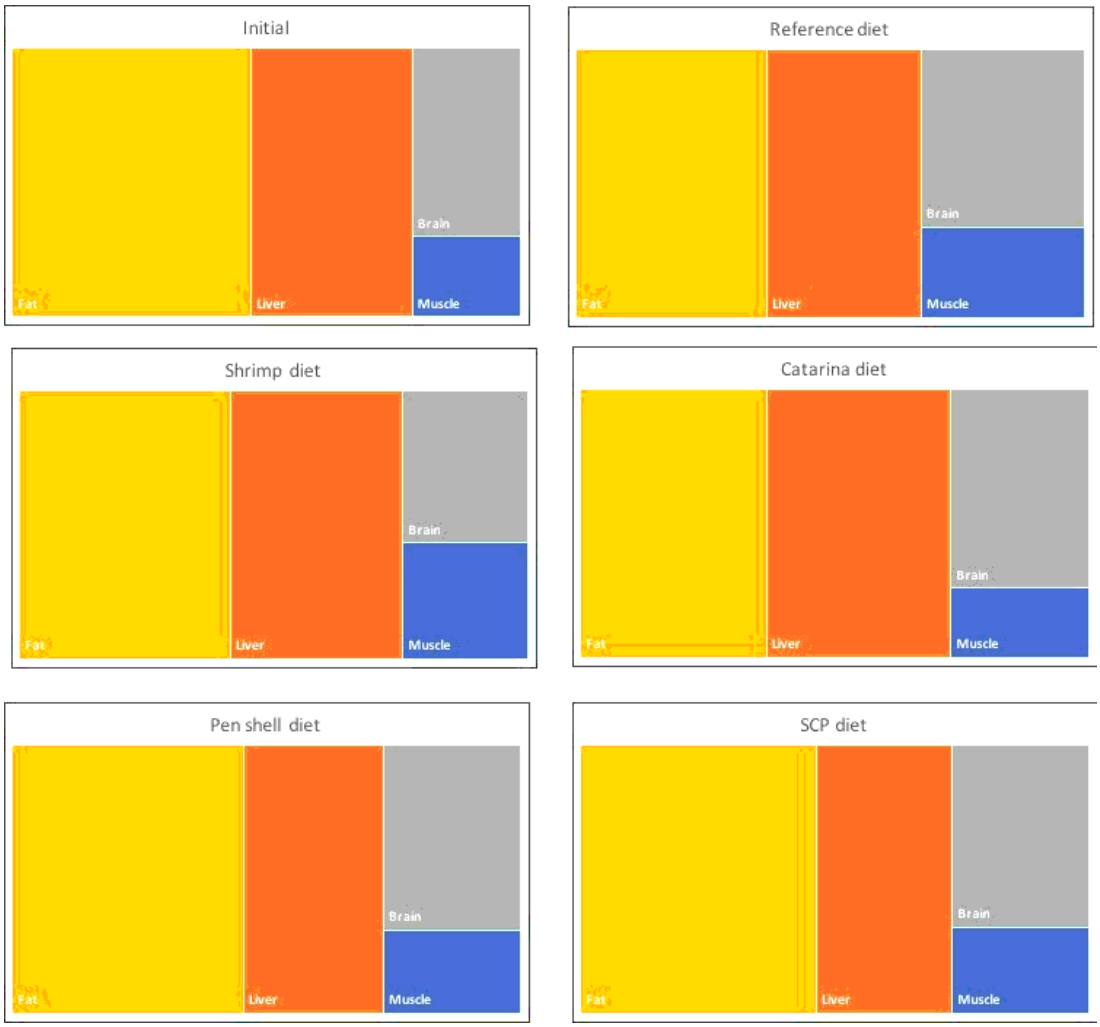

Figure 2

Concentration of 20:4n-6 (ARA, mg/g dw) content in muscle, liver, brain and mesenteric fat of S. rivoliana at the beginning of the bioassay (Initial) or after 60-d of feeding on a Reference diet; Shrimp head diet; Catarina scallop viscera diet; Pen shell viscera diet; and Shrimp head, Catarina and Pen shell viscera (mixed) diet (SCPD).

Supplementary Files

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