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Juvenile of Yellowtail, *Seriola dorsalis* fed diets from partial to total fish oil and fish meal replacement

Juveniles de jurel de Castilla, *Seriola dorsalis* alimentados con dietas con sustitución parcial a total del aceite y harina de pescado

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ABSTRACT | This study aimed to evaluate the effect of partial to total replacement of fish meal (FM) and fish oil (FO) from diets formulated for the yellowtail (*Seriola dorsalis*) using poultry by-product meal (PBM) as the main protein source, beef tallow as the main fat source, and supplemented with microalgae oil from *Schizochytrium* sp. Four experimental diets were formulated to be isoproteic (45.0 % crude protein) and isolipidic (12.0 % crude fat), based on the yellowtail nutritional requirements. Cholesterol was added to compensate its content in the FO. Lysine and methionine were also added to compensate its lower amount in the PBM. DHA-Nature™ was additional incorporated to reach similar levels of DHA than those present in FM and FO. One hundred and eighty *S. dorsalis* juveniles (14.54 g ± 0.19 g, mean ± SE) were randomly distributed into 12 tanks with 500 L each, connected into a recirculation system. After 48 days experimentation procedure, no significant differences were observed in performance. It is concluded that PBM can efficiently replace FM and FO in diets for *Seriola dorsalis* without any negative impact, where the fatty acid combination contained in the total replacement favored the use of fat as an energy source if DHA is enriched. However, it will be interesting to study the gene expression within the growth axis to fully comprehend the role of somatic growth vs. growth in length.

Palabras clave
Alimento libre de pescado
Sustentabilidad
Nutrición
Alimentos
Acuicultura

RESUMEN | Este estudio tuvo como objetivo evaluar el efecto de la sustitución parcial a total de la harina de pescado (FM) y el aceite de pescado (FO) de las dietas formuladas para el jurel de Castilla (*Seriola dorsalis*), utilizando harina de subproductos avícolas (PBM) como la principal fuente de proteína, y sebo de res como principal fuente de grasa suplementado con aceite de microalgas de *Schizochytrium sp*. Se formularon cuatro dietas experimentales isoproteicas (45,0% de proteína cruda) e isolipídicas (12.0% de grasa cruda), con base en los requerimientos nutricionales del jurel de Castilla. Se añadió colesterol para compensar su contenido en el FO. También se agregaron lisina y metionina para compensar su falta en el PBM. Se incorporó adicionalmente la harina de microalgas para alcanzar niveles similares de DHA que los presentes en FM y FO. Los juveniles de *S. dorsalis* se distribuyeron aleatoriamente (180 con 14,54 g ± 0.19 g, media ± SE) en 12 tanques de 500 L cada uno. Los cuales estaban conectados a un sistema de recirculación. Después de 48 días de experimentación, no se observaron diferencias significativas en el rendimiento. Se concluye que el PBM puede reemplazar eficientemente a FM y FO en dietas para *S. dorsalis* sin ningún impacto negativo, donde la combinación de ácidos grasos contenida en el reemplazo total favoreció el uso de grasas como fuente de energía si se enriquece con DHA. Sin embargo, será interesante estudiar la expresión génica dentro del eje de crecimiento para comprender mejor el papel del crecimiento en peso en comparación al crecimiento en longitud.

INTRODUCTION

Aquaculture is one of the worldwide fastest-growing food sectors, with an average annual growth of 5.8% between 2000-2016 (FAO 2018). Therefore, it is desired to assess the sustainability of the aquaculture systems. Consequently, there is a need to seek more environmentally sustainable aquafeeds to mitigate the adverse environmental impact associated with their production (Tacon and Metian 2008). By sustainability we mean a path driven by continuous improvement processes to create more resource-efficient products to maintain functional ecosystems to satisfy the continuous needs of human to be warrant through several generations (Johnston *et al*. 2007). Therefore, for the aquaculture sector to grow in the future years, one needs to stop using the marine resources and be competitive to start using others to cope with ingredients needs in quality and quantity to provide feeds into the sector.

From an environmental point of view, the use of marine resources and the efficiency in their utilization will warrant the increase in production management (Tacon and Metian 2008). For this to happen, it is important to avoid the release of pollutants, but also the increase of unused by-products. For this reason, it is crucial that fish oil (FO) and fish meal (FM) be replaced by using protein and oil sources from recycled by-products such as terrestrial proteins from husbandry animals (Parés *et al*. 2014; Badillo *et al*. 2014), or plant protein and oils (Bendiksen *et al*. 2011; Pratoomyot *et al*. 2011). Despite the total use of FM and FO has continued its upward consumption trends due to the general increase in world aquaculture production (Tacon and Metian 2008; Naylor *et al*. 2009), there is a significant tendency to reduce marine ingredients from aquafeeds. While there is a great variety of research works focus into the use of alternative ingredients, derived from plants and terrestrial animals (Forster *et al*. 2003; Davidson *et al*. 2016; Stone *et al*. 2005; Lazzarotto *et al*. 2018; Oliva-Teles *et al*. 2015), still, some species appear not to tolerate plant proteins or their blends, while others can tolerate the presence of animal by-products (Fuentes-Quesada *et al*. 2018; Viana *et al*. 2019; Fuentes Quesada *et al*. 2020). The results somehow contradictory, should be taken with caution, considering the digestion capacity from a great variety of fish species. Herbivores normally present a great acceptance of plant protein sources, while marine carnivores show a better performance with animal proteins. Additionally, a great performance can be obtained in marine carnivore fish by the usage of FO, since it contains a group of essential LC-PUFAs that must be provided, and they are not present in terrestrial plant or animal fats (DHA, EPA). Therefore, the dependency on FO is more difficult to cope. However, marine microalgae are shown to produce large amount of those LC-PUFAs (especially DHA and EPA) missing from most alternative to FO used in aquaculture (Tang *et al*. 2011; Mata-Sotres *et al*. 2018; Araújo *et al*. 2019).

Most nutritional studies are based on comparative results (overall performance) using several levels of different proteins to substitute FM and FO, or in some cases measuring digestibility vs. other protein sources (Castillo-López *et al*. 2016; Mata-Sotres *et al*. 2018). This is how both vegetable ingredients (soy, lupine, wheat, corn, potatoes, among others; with or without food additives) and from animal sources (blood meal, meat and bone meal, and poultry by-product meal), have been investigated performing great potential to partially replace FM (Keramat *et al*. 2014).

Badillo *et al*. (2014), in a partial to total replacement model, using stable isotopes with the *Totoaba macdonaldi*, reported a better performance when combined two parts of poultry by-product meal (PBM) with one of FM, while fish fed a diet with a total replacement, presented a poor performance. These authors conclude that the low performance from the total FM/FO replacement was particularly due to the lack of essential fatty acids (LC-PUFAs) in these alternative sources. Other studies (Steffens 2003) showed that PBM is suitable as a partial or fully replacement in diets for rainbow trout (*Oncorhynchus mykiss*), although the fully replacement required amino acid supplementation (mainly lysine and methionine). Likewise, they recommend it together with feather meal as an additive to form a suitable ingredient for FM replacement in diets (Psofakis *et al*. 2020).

Some other PBM studies reported that replacement of 75% to 100% of FM did not result in a negative impact on fish growth (Sabbagh *et al*. 2019). PBM could replace 75% of FM in diets for gilthead (*Sparus aurata*) juvenile without the necessity to supplement with additional amino acids (Nengas *et al*. 1999).

Whereas Karapanagiotidis *et al*. (2018), demonstrated that PBM could replace 50% of FM without compromising growth performance, feed use, and proximal composition in this same species.

In our lab we have been able to replace at high level the FM in shrimp diets using PBM combined with soybean meal (SBM), supplemented with EPA/DHA (from microalgae oil) and cholesterol, without reducing the overall performance compared to a diet containing fish source nutrients (Araújo *et al*. 2019). As stated before, there are fewer examples of carnivore fish with a total replacement of marine ingredients. Therefore, this work aims to evaluate the effect of partial to total FM and FO replacement from diets formulated for the yellowtail, *Seriola dorsalis* (Gill, 1863), using PBM as the main protein source and microalgae oil from *Schizochytrium* sp.

MATERIALS AND METHODS

Experimental diets

Four experimental diets were formulated to be isoproteic (450 g crude protein kg^{-1} diet) and isolipidic (120 g crude fat kg^{-1} diet), based on the yellowtail nutritional requirements (NRC 2011; García-Organista *et al*. 2019; Viana *et al*. 2019; Aguillon *et al*. 2020) (Table 1). As recommended by Guerra-Olvera and Viana (2015), cholesterol was added to compensate its content in the FO. Lysine and methionine were also added to compensate its lower amount in the PBM. DHA-Nature™ was additional incorporated to reach similar levels of DHA than those present in FM and FO. Fatty acid profile from the experimental diets is presented in Table 2.

Experimental feeds were manufactured at the Instituto de Investigaciones Oceanológicas (UABC, México) according to in-house protocols. Briefly, the main ingredients were ground to 0.8 millimeters (Inmimex M-400, México), sifted (Kemutek-Gardner K300, USA) and mixed using a vertical cutter-mixer (Robot Coupe, USA) until a homogenized meal was achieved. Micronutrients (a premix of vitamins and minerals, together with antioxidants and antimicrobials) were mixed for a minute (Robot Coupe R-60, USA), and then incorporated to the bulk meal. Fish oil was added and thoroughly mixed. Then water was incorporated, and the whole blend was mixed around 10 min. After that, the dough was immediately pelleted using a commercial-grade food grinder and dried at 60°C until >90% moisture was achieved (around 24 h). All feeds were kept cooled (4˚C) throughout the feeding trial. Diets are identified as Control, T-Low, T-Med, and T-Total, this last represent the total substitution (fish-free).

Table 1. Ingredient composition and proximate analysis, and amino acid content of diets formulated to contain 45% crude protein (CP) and 12% crude fat (CF), used for experimental diets to replace fishmeal and fish oil replaced by poultry by-product meal and bovine tallow with an algae extract (DHA source). Diets were fed to juvenile yellowtail, *Seriola dorsalis*, for 48 days. Amino acids were calculated from the ingredient content.

	TREATMENTS			
Ingredients	**Control**	**T-Low**	**T-Med**	**T-Total**
Fish meal [a]	21	14	7	0
Poultry by product meal [b]	22	29.3	36.6	44
Yeast by-product (77% CP)[c]	5	5	5	5
Soybean meal 42%[d]	4	4	4	4
Yeast by-product (48% CP)[e]	4	4	4	4
Gelatin[f]	6	6	6	6
Corn gluten [g]	4	4	4	4
Rovimix[h]	2	2	2	2
Stay C[i]	0.1	0.1	0.1	0.1
Taurine [j]	2	2	2	2
Beef tallow [k]	0	2.7	2.8	2.9
Starch [l]	21.6	21	20.9	20.7
Lysine [m]	1	1	1	1
Methionine [n]	0.4	0.4	0.4	0.4
DHA-Nature™ (24% DHA) [o]	1	2.80	3.40	4

Fish oil [p]	6	1.8	0.9	0
Sodium benzoate [q]	0.2	0.2	0.2	0.2
Choline chloride [q]	0.09	0.09	0.09	0.09
Cholesterol [r]	0	0.2	0.25	0.3
Proximal composition (% dry matter)				
Crude protein (%)	45.37	45.32	45.31	45.30
Crude fat (%)	12.08	12.10	12.09	12.11
Ash (%)	2.5	2.6	2.7	2.7
NFE (by difference)				
Aminoacid content (%)				
Methionine	0.94	0.91	0.88	0.84
Methionine+Cysteine	0.70	0.68	0.67	0.66
Cysteine	0.21	0.22	0.22	0.22
Lysine	2.55	2.58	2.59	2.61
Taurine	1.91	1.89	1.87	1.84
Threonine	1.06	1.04	1.02	1.00
Valine	1.28	1.25	1.21	1.17
Arginine	1.75	1.77	1.80	1.82
Tryptophan	0.19	0.17	0.15	0.13
Isoleucine	1.03	1.00	0.96	0.93
Leucine	1.74	1.71	1.68	1.65
Phenylalanine	0.94	0.92	0.90	0.88
Tyrosine	0.47	0.43	0.38	0.32

[a] Fishmeal from California sardine (68%CP); [b] Kindly donated by the North America Renderers Association; [c] Feed 77™ kindly donated by Feed 77; [d] Soybean meal 42% CP (Colpax, SA de CV, México); [e] kindly donated by ADM, México; [f] commercial grade, 85% CP; [g] INGREDION SA de CV, México, 65% CP; [h] a vitamin and mineral mixture from DSM; [i] StayC from DSM; [j] NUBIOT SA de CV, México; [k] Kindly donated by Grasas y Derivados de Tijuana; [l] Maicena™, México; [m] donated from ADM, Mexico; [n] Future Foods, México [o] kindly donated by ADM; [p] from California sardine, Mazatlán, México; [q] Sigma Aldrich; [r] kindly donated by Mitsui, Mexico.

Table 2. Fatty acid content of four different diets formulated to contain from partial to total substitution of fish meal and fish oil fed to juvenile yellowtail (*Seriola dorsalis*) for 48 days.

Fatty acids	**TREATMENTS**			
	Control	**T-Low**	**T-Med**	**T-Total**
14:0	6.96	7.42	8.19	8.41
16:0	22.60	24.15	24.53	24.62
16:1n-7	6.72	4.30	4.69	4.47
18:0	5.20	8.52	8.58	8.52
18:1n-9	18.94	28.41	25.53	26.39
18:1n-7	2.61	1.88	2.10	1.88
18:2n-6	10.35	12.38	11.26	11.52
18:3n-3	1.41	1.08	0.93	0.67
18:4n-3	0.91	0.17	0.25	0.10
20:0	0.83	0.50	0.44	0.29
20:2n-6	0.40	0.34	0.36	0.32
20:4n-6	1.52	0.88	0.94	0.89
20:5n-3	6.23	0.78	0.99	0.23
22:5n-3	1.12	0.37	1.03	0.24
22:6n-3	7.19	4.43	5.05	5.75
24:1n-9	0.48	0.23	0.39	0.26
Others	6.48	4.19	4.61	5.56
Total	**100**	**100**	**100**	**100**

Experimental Design and Feeding Trial

One hundred and eighty juvenile *S. dorsalis* (14.54 g ± 0.19 g, mean ± SE) kindly donated from a commercial marine finfish hatchery (Maricultivos Baja Sel, S.A. de C.V., Erendira, B.C., Mexico), were acclimated for two weeks and randomly stocked (15 fish tank^{-1}) into a recirculating aquaculture system. The experimental system was equipped with 12 tanks with 500-L each, with supplemental aeration, and biofilter. Water temperature was controlled and maintained steady with a heater, whereas the photoperiod was determined by natural conditions. Temperature, dissolved oxygen, and salinity were measured daily using a YSI-Pro 2030 optical multi-probe meter (YSI Inc., Yellow Springs, OH, USA). Total ammonia, nitrite, and nitrate-nitrogen were quantified three times per week using API test kits (Mars Fishcare Inc, Chalfont, PA, USA) and pH was measured weekly using a Thermo Scientific Orion 4-Star pH meter (Thermo Scientific, Waltham, MA, USA).

Water quality parameters were monitored and maintained within suitable ranges for this species: temperature = 21.5 ± 0.5 °C, salinity = 34.1 ± 0.2 psu, dissolved oxygen = 7.15 ± 0.14 mg L^{-1}, total ammonia nitrogen = 0.28 ± 0.08 mg L^{-}, nitrite-nitrogen = 0.97 ± 0.97 mg L^{-1}, nitrate-nitrogen = 46.7 ± 30.5 mg L^{-1}, and pH = 7.50 ± 0.08 (mean ± SE). Dietary treatments were randomly assigned to three replicate tanks. Fish were hand-fed three times daily to apparent satiation (08:00, 12:00, and 16:00) and tanks were cleaned daily using a siphon. Every two weeks partial biometrics was taken to adjust the feed ratio.

Sampling

Upon completion of the 48 days feeding trial, fish were counted and group-weighed by the tank to assess performance in terms of the following metrics:

Specific Growth Rate = SGR = (ln final weight –ln initial weight)*100/t
Thermal growth coefficient (TGC) = [(final weight ⅓ - initial weight ⅓) / (T◦C x days)] x 1000.
Feed Conversion Ratio (FCR) = total feed consumed / wet weight gained.
Protein Efficiency Ratio (PER) = weight gain / protein intake.
Feed Intake = FI (% day^{-1}) = 100 x (total amount of the feed consumed / ((initial body weight + final body weight) / 2) / days).

All fish were weighed and dissected to collect the following morphometric parameters:
HSI (Hepatosomatic Index) = (liver weight / body weight) x 100.
VSI (Viscerosomatic Index) = (viscera weight / body weight) x 100.

Analytical methods

To confirm proximate composition, all diets were analyzed in triplicate according to AOAC (1990; Table 1). Concisely, moisture content was gravimetrically determined by drying ground samples at 60˚C for 24 hours. Similarly, ash was determined by pulverizing ground sample in a muffle furnace at 550˚C for 6 hours. Crude protein was calculated by nitrogen conversion (%N x 6.25), nitrogen content was measured by the micro-Kjeldahl method followed by automatic distillation and titration (UDK 169, Velp, Italy). Lipid content was determined gravimetrically and extracted with Soxhlet method using petroleum ether as solvent. The amino acid profile was theoretically calculated from the amino acid content of each protein.

Different samples were used for fatty acid profile from fish, while feed samples were also extracted for fatty acid analysis. Samples were extracted from the fresh samples (muscle and liver tissues) or pellets, according to Folch *et al*. (1957), but using dichloromethane instead of chloroform. After that, the fatty acids were methylated following the method described by Parrish *et al*. (2015). Fatty acids methyl esters (FAME) were analyzed using gas chromatography equipped with flame ionization detector (Agilent GC 6880, Agilent Technologies, Santa Clara, CA, USA) using nitrogen as the carrier gas. The GC column (60 m x 0.25 mm with 0.25 µm film thickness; Agilent 122-2362 dB-23) conditions were: oven temperature initial of 50 ºC for 1 min, 50 to 140 °C at 30 ºC min^{-1}, held at 140 ºC for 5 min, from 140 to 240 ºC at 4 ºC min^{-1},

and finally 240 ºC for 20 min. The injector and detector temperatures were kept at 230 and 260 ºC, respectively. FAME were identified comparing retention times using different standards (37 Component FAME mix, PUFA 1 and PUFA 3, Supelco/Sigma-Aldrich, St. Louis, MO, USA).

Statistical analyses

Significant differences in performance indices and somatic indices, were analyzed by one-way ANOVA test, followed by post-hoc Tukey rank test. Also, a regression among treatments was performed. For all cases, statistical significance was set at $P<0.05$. Statistical analysis was performed using the software STATISTICA 8.0™ (StatSoft, Inc. USA).

RESULTS

Growth performance, feed utilization, and somatic indexes

The growth performance of *S. dorsalis* after 48 days feeding experiment is shown in Table 3. At the end of the trial, no clear significant differences were observed among treatments in all the biological index measured and calculated. Among them, the final weight and weight gain (%) resulted in similar results in fish from different treatments. Whereas only growth in length was significantly different with the highest length (15.42 cm) registered in fish from control group, otherwise the results in the other treatments were consistent (14.42 to 14.99 cm). The specific growth rate (SGR) was similar in fish from all treatments, while no differences were observed TGC. However, the fish free treatment (T-Low) resulted in a numerical lower growth than the control group (Control), but similar to T-Med, and T-High (2.02, 2,21, and 2.05 %/day as SGR). The weight gain (%) reached the 200% in the Control diet whereas the T-Total (fish-free) was only 168%, without showing significant differences among the experimental treatments (T-Low, T-Med, and T-Total). Mortality was not registered along the experimental procedure.

Table 3. Biological indices of juvenile yellowtail (*Seriola dorsalis*) fed for 48 days four different diets from partial to total substitution of fish meal and fish oil. Mean values and standard deviation (n=3) are given. Values in the same row with a different superscript were significantly different (P <0.05).

Biological indices	**TREATMENTS**				
	Control	**T-Low**	**T-Med**	**T-Total**	***P Value***
Initial weight (g)	14.51±0.02	14.30±0.14	14.77±0.26	14.55±0.18	0.122
Final weight (g)	43.48±1.41	37.90±2.17	42.88±9.95	39±1.82	0.065
Weight gain (%)	200.94±9.39	163.2±16.79	191.4±25.28	168.8±15.2	0.087
Total initial length (cm)	10.98±0.09	10.82±0.15	10.99±0.10	10.92±0.05	0.285
Total final length (cm)	15.42±0.154^{a}	14.42±0.174^{b}	14.99±0.516^{ab}	14.83±0.30^{a}	0.031
SGR (%/day)	2.28±0.06	2.02±0.13	2.21±0.22	2.05±0.11	0.170
TCG	1.01±0.05	0.82±0.08	0.98±0.14	0.85±0.07	0.096
FI (% day $^{-1}$)	2.95±0.11	3.09±0.02	2.99±0.10	2.95±0.29	0.723
FCR	1.39±0.06	1.61±0.08	1.45±0.16	1.52±0.10	0.177
PER	1.59±0.07	1.37±0.07	1.53±0.18	1.46±0.10	0.203
HIS	2.23±0.24	1.87±0.44	1.89±0.23	2.34±0.72	0.510
VIS	17.10±0.80	15.17±2.01	15.73±0.76	17.33±3.79	0.596
Survival (%)	100	100	100	100	
CF					

SGR = (ln final weight –ln initial weight)*100/t
TGC = [(final weight ⅓ - initial weight ⅓) / (T°C x days)] x 1000.
FCR = total feed consumed / wet weight gained.
Protein Efficiency Ratio (PER) = weight gain / protein intake.
FI = 100 x (total amount of the feed consumed / ((initial body weight + final body weight) / 2) / days).
HSI = (hepatopancreas weight / body weight) x 100.
VSI = (viscera weight / body weight) x 100.
Condition factor (CF) (g cm^3-1)= total fish weight (g) / $length^3$ (cm)

Proximal composition

It was observed a higher level of fat in muscle of fish from T-Low compared those from T-Total, while fish from T-Med and Control resulted in intermediate levels. Inversely, higher levels of protein were observed in the muscle tissue of fish from T-Total compared to fish from Control and T-Med. Ash content was most representative in fish from Control and T-Low compared to T-Med and T-Total treatments (Table 4). Fat levels were higher in liver of fish from Control compared those from T-Low and T-total, while ash levels were higher in Control and T-Low compared to T-Med (Table 5).

Table 4. Chemical composition (%) of muscle tissue of juvenile yellowtail (*Seriola dorsalis*) fed for 48 days four different diets from partial to total substitution of fish meal and fish oil. Mean values and standard deviation (n=3) are given.

	TREATMENTS				
	Control	**T-Low**	**T-Med**	**T-Total**	***P Value***
Crude fat	5.0±2.62^{ab}	6.72±0.10^{b}	6.48±0.20^{ab}	4.22±0.24^{a}	0.03
Crude protein	76.95±0.02^{c}	78.21±1.35^{bc}	78.62±0.24^{b}	83.19±00.83^{a}	0.001
Ash	7.36±0.47^{a}	7.23±0.25^{a}	6.06±0.25^{b}	6.84±0.34^{b}	0.001
Moisture	74.58±0.30	74.85±0.80	73.87±2.65	69.47±1.43	0.53

Values in the same row with a different superscript were significantly different (P <0.05).

Table 5. Chemical composition (%) of liver tissue of juvenile yellowtail (*Seriola dorsalis*) fed for 48 days four different diets from partial to total substitution of fish meal and fish oil. Mean values and standard deviation (n=2) are given.

	Dietary Treatments				
	Control	**T-Low**	**T-Med**	**T-Total**	***P Value***
Crude fat	8.48±5.69^{a}	2.62±1.49^{b}	5.92±0.03^{ab}	3.28±1.80^{b}	0.04
Ash	7.36±0.49 ^{a}	7.25±0.25 ^{a}	6.08±0.25 ^{a}	6.84±0.34^{ab}	0.007
Moisture*	69.45	70.84	71.28	72.44	-

* Due to the lack of sample material during proximal liver analyzes, triplicate sampling could not be performed and therefore an ANOVA was not performed.

Fatty acid profile

Significant differences were observed in the fatty acid profile of muscle tissue among treatments. The main differences observed were higher percentage of 14:0, 16:0, and EPA in muscle of fish from the Control group, and inversely, higher levels of 18:1n-9, 18:2n-6 and 22:5n3 in fish from the other experimental groups compared to the Control group. It was observed a lower DHA level in fish from T-Low compared to those from T-Med.

DISCUSION

The present work showed that it is possible to fully replace FM and FO from diets for yellowtail juveniles, since no significant differences were observed after 48 experimental days on the overall performance among fish from different treatments. However, fish from the control group reached a higher length compared those from the other treatments. Growth in weight in the total substitution was numerically lower as well, but without reaching significant differences, similarly to the condition index, protein efficiency ratio, and all different biological indexes evaluated. This result could implicate that growth in the long term could result in significant differences. However, it is essential to state that ash content from the chemical composition of the whole body from fish at the end of the experiment was lower in T-Total dietary treatment. Low ash content within an equal growth rate means that at a similar weight, higher flesh is involved within similar circumstances. It will be challenging in the future to investigate the role of

different genes involved in the growth axis interaction, such as mTOR, GH, IGF, among others, to see what genes are upregulated in the full FM and FO replacement.

The formulated diets resulted in similar proximal composition, both in crude protein and fat. However, differences were observed in fatty acid profile, according to the crude fat source, considering that PBM contained 14% CF which is lower than FM. Differences mainly for the oleic acid (18:1n-9), a monosaturated fatty acid increasing from 18.9 to 26.4%. Also, the myristic acid (14:0) and palmitic (16:0) were from 6.9 to 8.4% and 22.6 to 24.6%, respectively. It is known that SFA and MUFA are efficiently catalyzed as energy sources not only by *S. dorsalis* but generally by most fish species (Tocher 2003), whereas the PUFA are used afterward, the LC-PUFA, especially DHA, are generally retained (Tocher 2015). Here, even if diets were supplemented with DHA-Nature™ to compensate for the DHA in a fish reach diet, the DHA and particularly the EPA were gradually reduced. Accordingly, FM and FO were substituted. However, DHA was retained in muscle tissue and showed a similar content across the different dietary treatments. Also, EPA was efficiently retained considering its low content in the diet with total FO replacement, similarly to that observed for 20:4n-6 (arachidonic acid - ARA).

Interestingly, the liver chemical composition varied drastically, reducing more than 50% of total fat content when FO and FM were gradually reduced. This result means that crude fat from beef tallow supplemented with DHA is efficiently catalyzed, whereas the LC-PUFAs are efficiently retained (LC-PUFA sparing effect). The higher availability of fat as an energy source contributed to reaching the protein-sparing effect resulting in higher somatic growth in those fish fed the T-Total dietary treatment as stated before and proven for the lower ash content. On the other hand, the T-Total dietary treatment failed to show a similar amount of DHA, and the EPA was much lower (7.19 and 6.23 vs. 5.75 and 0.23% from the Control and T-Total respectively). However, fish resulted in a similar growth rate and protein efficiency ratio (PER) despite this difference. Furthermore, notwithstanding the lower DHA and EPA contents in diets, the retention in muscle tissue failed to show significant differences in DHA while EPA was efficiently retained reaching a 1.4% in the muscle tissue from the 0.23% content in the diet. The latter implies that even though the DHA in the diet was low, the microalgae oil (DHA-Nature™) was more efficiently retained than DHA from the FO. Similarly, the EPA decreased as FO was reduced. However, the EPA reduction was directly reflected in the muscle content of *S. dorsalis* at the end of the experiment. Similarly, in an earlier experiment with cobia juveniles (*Rachycentron canadum*), DHA was also supplemented but failed to show an accumulation in the muscle tissue and dietary treatments with or without DHA supplementation for FO (Marques *et al*. 2021).

In contrast, fatty acids in dietary treatments showed an apparent increase in oleic acid (18:1n-9), as well as a decrease in linolenic (18:2n-3) and ARA (1.52 to 0.89%). Despite these differences, the fatty acids detected in the muscle at the end of the experimentation showed ARA levels had a reduction from 3.2 to 2.04% (Control vs. T-Total dietary treatments) as shown in Table 6. ARA can be converted into bioactive eicosanoids such as prostaglandins, leukotriene, and prostanoids (Smith 1992) and even reduce cortisol (Koven *et al*. 2001; Tharuka *et al*. 2019; Chee *et al*. 2020), whereas EPA seems to be somewhat expandable for marine fish species (Trushenski *et al*. 2012). However, studies are revealing a necessary ARA/EPA ratio (Norambuena *et al*. 2016). However, in the present work, even if different amounts of ARA were encountered in diets without any enrichment, the T-Total dietary treatment revealed an amount of 0.89%, considered as optimum (unpublished work). In the same work, it was found that ARA has a strong influence on the fatty acid profile of liver and muscle tissue. Therefore, we assume that the total replacement of FM with PBM lasted in a proper fatty acid profile of ARA.

When the SFA:MUFA ratio increased (12:0 and 14:0) in *S. dorsalis* dietary treatments (Mata-Sotres *et al*. 2021), it revealed that DHA at a lower amount in diets (8.91 in the Control group, vs. 2.71-2.74 in the experimental treatments), the SFA were not accumulated showing a preferential use as an energy source, while the DHA never reach similar retention than in the Control group fed FO. Intriguing, in the same experiment, the 18:1n-9 increased along the PBM substitute the FM, from 19.9 to 26.4% along with the diets. In contrast, the fatty acid accumulation shows that oleic acid was efficiently utilized with a final accumulation of 11.6 to 20.1% in the different dietary treatments. Although we could not measure the fatty

acid content in liver tissue, since total crude fat in the liver was reduced by more than 50%, one can assume that lipids were efficiently catabolized.

Despite 18:1n-9, increased along with the PBM content (18 to 26%). The fish muscle tissue accumulated between 11.6 to 20.1%, almost reflecting its content in subsistence quantity. Previous work has shown that FO replaced by poultry oil, rich in 18:1n-9 in *Seriola lalandi* juveniles, resulted in a proper energy source even at lower temperatures (Bowyer *et al*. 2012). Further, even if 18:2n-6 did not vary substantially in diets, it was accumulated in muscle tissue, where a 14.7% was found in the muscle tissue from fish fed T-Total dietary treatment while in the Control diet, only a 7.5% could be revealed. Similarly, Bowyer *et al*. (2012) observed where 18:2n-6 is accumulated when fed with poultry oil, whereas a much higher accumulation was seen when canola oil was used.

It is concluded that PBM can efficiently replace the FM and FO in diets for *S. dorsalis* without any negative impact in performance, where the fatty acid combination contained in the total replacement favored the use of fat as an energy source if DHA is enriched. However, it will be interesting to study the gene expression within the growth axis to fully comprehend the role of somatic growth vs. growth in length.

Table 6. Fatty acid content in the muscle tissue of juvenile yellowtail (*Seriola dorsalis*) fed for 48 days four different diets from partial to total substitution of fish meal and fish oil. Mean values and standard deviation (n=3) are given.

Fatty acids	**TREATMENTS**				
	Control	**T-Low**	**T-Med**	**T-Total**	***P Value***
14:0	3.38±0.87^{b}	2.29±0.10^{a}	1.98±0.59^{a}	2.10±0.51^{a}	0.01
16:0	17.20±0.00^{a}	16.64±0.23^{a}	15.33±0.23^{b}	15.16±1.12^{b}	0.007
16:1n-7	3.58±0.21^{c}	2.46±0.12^{ab}	2.26±0.18^{a}	2.84±0.51^{ab}	0.001
18:0	8.84±0.03	9.08±0.31	8.49±0.17	9.32±1.58	0.380
18:1n-9	11.61±0.27^{b}	18.63±0.78^{a}	17.65±0.41^{a}	20.10±4.08^{a}	0.001
18:1n-7	3.72±0.19^{c}	3.07±0.22^{b}	2.63±0.23^{b}	1.53±0.51^{a}	0.001
18:2n-6	7.51±0.28^{c}	14.77±1.07^{a}	13.18±0.35^{b}	14.70±1.14^{a}	0.001
18:3n-3	1.41±0.05^{a}	1.05±0.03^{bc}	0.93±0.35^{ab}	0.49±0.38^{c}	0.001
18:4n-3	1.05±0.10^{b}	0.34±0.07^{a}	0.28±0.08^{a}	0.40±0.27^{a}	0.001
20:0	1.53±0.20^{a}	1.04±0.05^{b}	0.84±0.04^{ab}	0.76±0.09^{c}	0.001
20:2n-6	0.78±0.09^{a}	0.51±0.16^{b}	0.64±0.03^{ab}	0.63±0.13^{ab}	0.250
20:4n-6	3.20±0.18^{a}	2.12±0.27^{c}	2.65±0.20^{b}	2.04±0.10^{c}	0.001
20:5n-3	6.53±0.30^{a}	2.96±0.28^{b}	3.02±0.37^{b}	1.40±0.63^{c}	0.001
22:5n-3	2.26±0.11^{a}	2.48±0.17^{b}	4.20±0.04^{c}	4.72±0.03^{d}	0.001
22:6n-3	20.90±0.48^{ab}	18.29±0.69^{b}	21.81±1.43^{a}	20.38±3.24^{ab}	0.050
Others	6.49^{a}	4.26^{c}	4.12^{bc}	3.26^{c}	0.001
Total	**100**	**100**	**100**	**100**	

Values in the same row with a different superscript were significantly different (P <0.05).

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

Aguillón, O., Mata, J.A., D'Abramo, L.R., Viana, M.T. (2020). Lipid metabolism in juveniles of Yellowtail, *Seriola dorsalis* fed different levels of dietary methionine containing a low level of cholesterol: Implication in feed formulation. *Aquaculture Nutrition*. 26(5), 1463-1475.

AOAC (2005). Official Methods of Analysis. Association of Analytical Chemists, Arlington, VA, USA.

Araújo, B., Honji, R.M., Rombenso, A.N., Souza, G.B., Mello, P.H., Hilsdorf, A.W.S., Moreira, R.G. (2019). Influence of different arachidonic acid levels and temperatura on the growth performance, fatty acid profile, liver morphology and expression of lipid genes in cobia (*Rachycentron canadum*) juveniles. *Aquaculture* 511, 734245. https://doi.org/10.1016/j.aquaculture.2019.734245.

Badillo-Zapata, D., Lazo, J.P., Herzka, S., Viana, M.T. (2014). The effect of substituting fishmeal with poultry by-product meal in diets for *Totoaba macdonaldi* juveniles. *Aquaculture Research*. 47.

Bendiksen, E.Å., Johnsen, C.A., Olsen, H.J., Jobling, M. (2011). Sustainable aquafeeds: progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar L.*). *Aquaculture* 314 (1-4), 132–139.

Castillo-López, E., Espinoza-Villegas, R.E., Viana, M.T. (2016). In vitro digestion comparison from fish and poultry by-product meals from simulated digestive process at different times of the Pacific Bluefin tuna, *Thunnus orientalis*. *Aquaculture* 458, 187-194.

Chee, W.L., Turchini, G.M., Teoh, C.Y., Ng, W.K. (2020). Dietary arachidonic acid and the impact on growth performance, health and tissues fatty acids in Malabar red snapper (*Lutjanus malabaricus*) fingerlings. *Aquaculture* 519, 734757. doi: 10.1016/j.aquaculture.2019.734757.

Davidson, J., Barrows, F.T., Kenney, P.B., Good, C., Schroyer, K., Summerfelt, S.T. (2016). Effects of feeding a fishmeal-free versus a fishmeal-based diet on post-smolt Atlantic salmon *Salmo salar* performance, water quality, and waste production in recirculation aquaculture systems. *Aquacultural Engineering* 74, 38–51.

FAO, 2018. The State of World Fisheries and Aquaculture, 2018. Food & Agriculture Org.

Folch, J., Lees, M., Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 276, 497–507.

Forster, I., Dominy, W., Obaldo, L., Tacon, A.G.J. (2003). Rendered meat and bone meals as ingredients of diets for shrimp *Litopenaeus vannamei* (Boone, 1931). *Aquaculture* 219 (1-4), 655–670.

Fuentes-Quesada, J.P., Rombenso, A.N., Viana, M.T., Guerrero-Rentería, Y., Lazo, J.P., Mata-Sotres, J.A. (2018). Enteritis induction by soybean meal in *Totoaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity. *Aquaculture* 495, 78-89. Doi: 10.1016/j.aquaculture.2018.05.025 ISSN: 0044-8486.

Fuentes-Quesada, J.P., Cornejo-Granados, F., Mata-Sotres, J.A., Ochoa-Romo, J.P., Rombenso, A.N., Guerrero-Rentería, Y., Lazo, J.P., Pohlenz, C., Ochoa-Leyva, A., Viana, M.T. (2020). Prebiotic agavin in juvenile totoaba, *Totoaba macdonaldi* diets, to relieve soybean meal-induced enteritis: Growth performance, gut histology, and microbiota. *Aquaculture Nutrition* 26(6), 2115-2134.

García- Organista, A., Mata-Sotres, J.A., Viana, M.T., Rombenso, A.N. (2019). The effects of high dietary methionine and taurine are not equal in terms of growth and lipid metabolism of juvenile California Yellowtail (*Seriola dorsalis*). *Aquaculture* 512 734-304.

Guerra-Olvera, F.M., Viana, M.T. (2015). Effect of dietary cholesterol content on growth and its accumulation in liver and muscle tissues of juvenile yellowtail kingfish (*Seriola lalandi*). *Ciencias Marinas*, 41(2):143-156 doi.org/10.7773/cm.v41i2.2514.

Johnston, P., Everard, M., Santillo, D., Robert, K.H. (2007). Reclaiming the definition of sustainability.

Environmental Science and Pollution Research International 14, 60–66.

Karapanagiotidis, I.T, Psofakis, P., Mente, E., Malandrakis, E., Golomazou, E. (2018). Effect of fishmeal replacement by poultry by-product meal on growth performance, proximate composition, digestive enzyme activity, haematological parameters and gene expression of gilthead seabream (*Sparus aurata*). *Aquaculture Nutrition* 10.1111/anu.12824.

Keramat, A.A., Shahsavary, M., Hedayatifard, M. (2014). Full Replacement of Fishmeal by Poultry by–Product Meal in Rainbow Trout, *Oncorhynchus mykiss* (Walbaum, 1972) Diet. *Iranian Journal of Fisheries Sciences* 13. 1069-1081.

Koven, W., Barr, Y., Lutzky, S., Ben-Atia, I., Weiss, R., Harel, M., Behrens, P., Tandler, A. (2001). The effect of dietary arachidonic acid (20,4n−6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. *Aquaculture* 193, 107–122. Doi: 10.1016/S0044-8486(00)00479-8.

Lazzarotto, V., Médale, F., Larroquet, L., Corraze, G. (2018). Long-term dietary replacement of fishmeal and fish oil in diets for rainbow trout (*Oncorhynchus mykiss*): Effects on growth, whole body fatty acids and intestinal and hepatic gene expression. *PLoS One* 13 (1), e0190730.

Marques, V.H., Moreira, R.G., Branco, G.S., Honji, R.M., Rombenso, A.N., Viana, M.T., Mello, P.H., Mata-Sotres, J.A., Araújo, B.C. (2021). Different saturated and monounsaturated fatty acids levels in fish oil-free diets to cobia (*Rachycentron canadum*) juveniles: Effects in growth performance and lipid metabolism. *Aquaculture* 541, 736843. Doi: 10.1016/j.aquaculture.2021.736843.

Mata-Sotres, J.A., Tinajero-Chavez, A., Barreto-Curiel, F., Pares-Sierra, G., Del Rio-Zaragosa, O.B., Viana, M. T., Rombenso, A.N. (2018). DHA (22:6n-3) supplementation is valuable in Totoaba macdonaldi fish oil-free feeds containing poultry by-product meal and beef tallow. *Aquaculture* 497, 440-451.

Mata-Sotres, J.A., Marques, V.H., Barba, D., Braga, A., Araújo, B., Viana, M.T., Rombenso, A.N. (2021). Increasing dietary SFA:MUFA ratio with low levels of LC-PUFA affected lipid metabolism, tissue fatty acid profile and growth of juvenile California Yellowtail (*Seriola dorsalis*). *Aquaculture* 543, 737011. doi: 10.1016/j.aquaculture.2021.737011.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliot, M., Farrell, A.P., Foster, I., Gatlin, D.M., Goldburg, R.J., Hua, K., Nichols, P.D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences of the United States of America* 106 (36), 15103–15110. Doi: 10.1073/pnas.0905235106.

Nengas, I., Alexis, M., Davies, S.J. (1999). High inclusion levels of poultry meals and related by-products in diets for gilthead seabream *Sparus aurata L*. *Aquaculture* 179, 13–23.

Norambuena, F., Rombenso, A., Turchini, G.M. (2016). Towards the optimization of Atlantic salmon reared at different water temperatures via the manipulation of dietary ARA/EPA ratio. *Aquaculture* 450, 48–57.

Oliva-Teles, A., Enes, P., Peres, H. (2015). Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. Feed and Feeding Practices in Aquaculture. Elsevier, pp. 203–233.

Parés-Sierra, G., Durazo, E., Ponce, M.A., Badillo, D., Correa-Reyes, G., Viana, M.T. (2014). Partial to total replacement of fishmeal by poultry by-product meal in diets for juvenile rainbow trout (*Oncorhynchus mykiss*) and their effect on fatty acids from muscle tissue and the time required to retrieve the effect. *Aquaculture Research* 45(9):1459-1469. doi:10.1111/are.12092 (ISSN: 1365-2109)

Parrish C.C., Nichols, P.D., Pethybridge, H., Young, J.W. (2015). Direct determination of fatty acids in fish tissues: quantifying top predator trophic connections. *Methods* 177, 85–95. https://doi.org/10.1016/0952-3278(90)90017-f.

Pelletier, N., Tyedmers, P. (2007). Feeding farmed salmon: Is organic better? *Aquaculture* 272 (1-4), 399–416.

Pratoomyot, J., Benediksen, E.A., Campbell, P.J., Jauncey, K.J., Bell, J.G., Tocher, D.R. (2011). Effects of different blends of protein sources as alternaives to dietary fishmeal on growth performance and body lipid composition of Atlantic salmon (*Salmo salar L.*). *Aquaculture* 316, 44-52.

Psofakis, P., Karapanagioditis, I.T., Malandrakis, E.E., Golomazou, E., Exadactylos, A., Mente, E. (2020). Effect of fishmeal replacement by hydrolyzed feather meal on growth performance, proximate composition, digestive enzyme activity, hematological parameters and growth-related gene expression of gilthead seabream (*Sparus aurata*). *Aquaculture* 521, 735006.

Smith, W.L. (1992). Protanoids biosynthesis and mechanisms of action. American Journal of Physiology 263, F181-F191.

Tharuka, M.D.N., Priyathilaka, T.T., Kim, J., Lim, C., Lee, J. (2019). Molecular characterization of big-belly seahorse (*Hippocamus abdominalis*) arachidonate 5-lipoxygenase (HaALOX5): First evidence of an immune defensive role by induced immunological stress in teleost. *Fish Shellfish Immunology* 86, 230–238. doi:10.1016/j.fsi.2018.11.042.

Trushenski, J., Schwarz, M., Bergman, A., Rombenso, A., Delbos, B. (2012). DHA is essential, EPA appears largely expendable, in meeting the n-3 long-chain polyunsaturated fatty acid requirements of juvenile cobia *Rachycentron canadum*. *Aquaculture* 326-329, 81–89.

Nengas, I., Alexis, M.N., Davies, S.J. (1999). High inclusion levels of poultry meals and related byproducts in diets for gilthead seabream *Spaus aurata* L. *Aquaculture* 179:1-4, 13-23.

Sabbagh, M., Schiavone, R., Brizzi, G., Sicuro, B., Zilli, L., Vilella, S. (2019). Poultry by-product meal as an alternative to fish meal in the juvenile gilthead seabream (*Sparus aurata*) diet. *Aquaculture*, 511, 734220.

Steffens, W. (2003). Replacing fish meal with poultry by-product meal in diets for rainbow trout, Oncorhynchus mykiss. *Aquaculture* 124, 27-34.

Stone, D.A.J., Hardy, R.W., Barrows, F.T., Cheng, Z.J. (2005). Effects on Extrusion on Nutritional Value of Diets Containing Corn Gluten Meal and Corn Distiller´s Grain for Rainbow Trout, *Oncorhynchus mykiss*. *Journal of Applied Aquaculture* 17 (3), 1–20.

Tacon, A.G., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285 (1-4), 146–158.

Tang, S., Qin, C., Wang, H., Li, S., Tian, S. (2011). Study on supercritical extraction of lipids and enrichment of DHA from oil-rich microalgae. *Journal of Supercritical Fluids* 57, 44-49.

Tlusty, M.F., Thorsen, Ø. (2017). Claiming seafood is 'sustainable' risks limiting improvements. *Fish and Fisheries* 18 (2) (2017) 340–346, doi.org/10.1111/faf.12170.

Tocher, D.R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11 (2), 107–184. https://doi.org/10.1080/713610925.

Tocher, D.R. (2015). Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. *Aquaculture* 449, 94-107. doi:10.1016/j.aquaculture.2015.01.010.

Viana, M.T., Rombenso, A.N., Del Rio-Zaragoza, O.B., Nomura, M., Díaz-Arguello, R., Mata-Sotres, J.A. (2019). Intestinal health impairment of the California Yellowtail, *Seriola dorsalis*, using soybean meal in the diet. *Aquaculture* 513, 734443.

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