Efecto de la sustitución de la harina y el aceite de pescado por fuentes vegetales y animales en la alimentación de la seriola (*Seriola dumerili.* Risso, 1810)

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GRUPO DE ACUICULTURA Y BIODIVERSIDAD

Efecto de la sustitución de la harina y el aceite de pescado por fuentes vegetales y animales en la alimentación de la seriola (*Seriola dumerili.* Risso, 1810)

Effect of fish meal and fish oil substitution by vegetable and animal sources in the diet of yellowtail (*Seriola dumerili.* Risso, 1810)

Si te lo propones, puedes conseguirlo todo.

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Resumen

La *Seriola dumerili* (Risso, 1810) se presenta como una especie emergente en la acuicultura, con una buena adaptación a la cautividad y un rápido crecimiento. Sin embargo, apenas se dispone de información acerca de la composición adecuada de los piensos para esta especie, lo que es de especial importancia, dado que muchos de los ingredientes óptimos, como las harinas y aceites de pescado, se consideran recursos limitados, tanto desde el punto de vista ambiental como económico.

En la presente tesis doctoral, se llevaron a cabo diferentes experimentos en *Seriola dumerili*, con el objetivo de evaluar el efecto de la sustitución de la harina de pescado y el aceite de pescado en el crecimiento, parámetros nutritivos, eficiencia del crecimiento y calidad del producto final.

Los experimentos se iniciaron con una prueba para determinar la digestibilidad de fuentes proteicas alternativas para su inclusión en dietas para la seriola, con el objetivo de comprobar su idoneidad para esta especie, permitiendo en las siguientes pruebas formular los piensos en base a los coeficientes digestibles. Se ensayon 12 materias primas, vegetales y animales (harinas de: habas, camelina, soja, guisante, girasol, trigo, krill, krill desengrasado, pollo, calamar y pescado). Las proteínas animales fueron las que resultaron más adecuadas para la especie, y las vegetales por sí mismas no ofrecían un buen balance nutricional, aunque combinadas con otras fuentes proteicas, podían ser consideradas un ingrediente proteico.

Posteriormente, se llevó a cabo un experimento de sustitución de la harina de pescado por una mezcla de fuentes proteicas animales y vegetales. El experimento se inició con peces de 39 g y se finalizó con un peso medio de 365 g tras 154 días. Se ensayaron cuatro niveles de sustitución de la harina de pescado (0%, 33%, 66% y 100%). De los resultados de esta prueba se concluyó que la sustitución total de la harina de pescado no es posible para esta especie, ya que el crecimiento fue mucho menor, debido principalmente a la menor digestibilidad de los aminoácidos esenciales, así como de la energía,

además con este pienso se observó una mayor mortalidad. Con la sustitución del 66% se obtuvieron buenos resultados de crecimiento, eficiencia nutritiva y una elevada supervivencia.

A partir de los resultados obtenidos en la primera prueba de fuentes proteicas, se inició un segundo experimento con peces de 530 g (los peces fueron alimentados con los mismos piensos durante todo el periodo de crecimiento) y finalizó tras 84 días con un peso medio de 850 g de peso medio. Se ensayaron los tres piensos de la prueba anterior que mejores resultados habían proporcionado (0%, 33%, 66% de sustitución de harina de pescado), con el objetivo de estudiar el efecto de éstos en la calidad de la carne (perfil de ácidos grasos, metales pesados, análisis sensorial y organoléptico) de peces a talla comercial. Al final del experimento, se observó una reducción en la mayor parte de los ácidos grasos a medida que aumentaba la sustitución. En cuanto al nivel de metales pesados en el músculo, éstos no excedieron en ningún caso los valores recomendados por la UE. Algunos de los parámetros fisicoquímicos del filete sí se vieron afectados, pero no se reflejó en el análisis organoléptico, donde los jueces no fueron capaces de apreciar diferencias entre dietas, excepto en el color.

En el siguiente experimento se estudió el efecto de la sustitución del aceite de pescado por una mezcla de aceites vegetales (utilizando una mezcla de aceite de palma y aceite de linaza en proporción 4:1). La fase de crecimiento se inició con peces de 39 g y se finalizó con 390 g de peso medio tras 154 días de experimento. Se ensayaron tres niveles de sustitución (0%, 50% y 100% del aceite de pescado). Se observó que la mezcla de aceites vegetales puede ser utilizada para sustituir completamente el aceite de pescado en juveniles de *Seriola dumerili* sin afectar al crecimiento ni la utilización del alimento. Asimismo, el perfil de ácidos grasos esenciales en la dieta cubrió las necesidades de los juveniles de la seriola sin afectar a la salud de los peces, ni a las características nutricionales del pescado.

De los resultados de la presente tesis, se confirma que, la sustitución de harina de pescado en piensos por la mezcla de fuentes proteicas alternativas ensayada, no afecta negativamente al crecimiento de la

seriola en periodos largos de alimentación, así como a parámetros de calidad como el perfil de ácidos grasos del filete, cuando dichas sustituciones son menores del 66%. En cuanto a la sustitución lipídica, la sustitución completa de aceite de pescado por aceites vegetales es posible en juveniles de *S. dumerili* sin afectar al crecimiento, rendimiento, utilización del alimento y salud de los peces, lo que deberá de tenerse en cuenta en futuras formulaciones de piensos específicos para esta especie.

Resum

La *Seriola dumerili* (Risso, 1810) es presenta com una espècie emergent en l'aqüicultura, amb bona adaptació a la captivitat i un ràpid creixement, però actualment no es disposa de molta informació sobre la composició adequada dels pinsos per a aquesta espècie, la qual cosa és d'especial importància donat el fet que molts dels ingredients òptims, com les farines i olis de peix, es consideren recursos limitats, tant des del punt de vista ambiental com econòmic.

En la present tesi doctoral, es van a dur terme diferents experiments en *Seriola dumerili*, amb l'objectiu d'avaluar l'efecte de la substitució de la farina de peix i l'oli de peix en el creixement, paràmetres nutritius eficiència del creixement i qualitat del producte final.

Els experiments es van iniciar amb una proba per a determinar la digestibilitat amb fonts proteiques alternatives per a la inclusió de dietes en la seriola, amb l'objectiu de comprovar la seua idoneïtat per a aquesta espècie permitint en les següents probes formular els pinsos sobre la base dels coeficients digestibles. Es van assajar 12 matèries primeres, vegetals i animals (farines de: faves, camelina, soja, pésol, gira-sol, blat, krill, krill desgreixat, au, calamar i peix). Les proteïnes animals van ser les que van resultar més adequades per a l'espècie i les vegetals per si mateixes no oferien un bon balanç nutricional, encara que combinades amb altres fonts proteiques, podien ser considerades un ingredient proteic.

Posteriorment, es va dur a terme un experiment de substitució de la farina de peix per una mescla de fonts proteiques animals i vegetals. L'experiment es va iniciar amb peixos de 39g i es va finalitzar amb una pes mig de 365 g després de 154 dies. Es van assajar quatre nivells de substitució de la farina de peix (0%, 33%, 66% i 100%). Dels resultats d'aquesta prova es va concloure que la substitució total de la farina de peix no és possible per a aquesta espècie, ja que el creixement va ser molt menor, degut principalment a la menor digestibilitat dels aminoàcids essencials, així com de l'energia, a més amb aquest pinso va haver-hi una major mortalitat. Amb la substitució del 66% es va

obtindre un bon resultat de creixement, eficiència nutritiva i una alta supervivència.

A partir dels resultats obtinguts en la primera prova de fonts proteiques es va iniciar un segon experiment amb peixos de 530 g (els peixos van ser alimentats amb els mateixos pinsos durant tot el període de creixement) i va finalitzar després de 84 dies amb una grandària de 850 g de pes mitjà. Es van assajar els tres pinsos de la prova anterior que millors resultats havien proporcionat (0%, 33%, 66% se substitució de farina de peix), amb l'objectiu d'estudiar l'efecte d'aquests mateixos pinsos en la qualitat de la carn (perfil d'àcids greixos, metalls pesats, anàlisi sensorial i organolèptic) en peixos aconseguint una talla comercial. Al final de l'experiment, es va observar una reducció en la major part dels àcids greixos a mesura que augmentava la substitució. En quant al nivell de metalls pesats en el múscul, aquests no van excedir en cap cas els valors recomanats per la UE. Alguns dels paràmetres fisicoquímics del filet sí que es van veure afectats, però no es va reflectir en l'anàlisi organolèptic, on els tastadors no van ser capaços d'apreciar diferències entre dietes, excepte en el color.

En el següent experiment es va estudiar l'efecte de la substitució de l'oli de peix per una mescla d'olis vegetals utilitzant una mescla d'oli de palma i oli de llinosa en proporció 4:1). L'experiment es va iniciar amb peixos de 39 g i es va finalitzar amb 390 g de pes mitjà després de 154 dies d'experiment. Es van assajar tres nivells de substitució (0%, 50% i 100% de l'oli de peix). Es va observar que la mescla d'olis vegetals pot ser utilitzada per a substituir completament l'oli de peix en juvenils de *Seriola dumerili* no va afectar el creixement ni la utilització de l'aliment. Així mateix, el perfil d'àcids greixos essencials en la dieta, va cobrir les necessitats dels juvenils de la seriola sense afectar la salut dels peixos ni a les característiques nutricionals del peix.

Dels resultats de la present tesi, es confirma que, en períodes llargs d'alimentació la substitució de farina de peix per la mescla de fonts proteiques alternatives assajada en pinsos, no afecta negativament al creixement de la seriola i a paràmetres de qualitat com el perfil d'àcids

greixos del filet, quan aquestes substitucions són menors del 66%. Quant a la substitució lipídica, la substitució completa d'oli de peix per olis vegetals és possible en juvenils de *S. dumerili* sense afectar el creixement, rendiment, utilització de l'aliment i salut dels peixos, la qual cosa haurà de tindre's en compte en futures formulacions de pinsos específics per a aquesta espècie.

Abstract

Seriola dumerili (Risso, 1810) is presented as a emerging specie in aquaculture, with a good adaptation to captivity and a rapid growth, but actually but not much information is currently available on the optimal feed composition for this specie. This is especially important since many of the optimal ingredients, such as fishmeal and fish oils, are considered limited resources from an environmental and economic point of view.

In the present Ph. D thesis, different experiments were carried out in *Seriola dumerili*, with the objective of evaluate the effect of fish meal ad fish oil substitution on growth, nutritional parameters, growth efficiency and the quality of the final product.

Experiments with alternative protein sources were initiated with a digestibility test of raw materials recommended for the *Seriola dumerili*, in order to check their suitability for this specie and permiting in the next experiments formulate the diets according the obtained results. 12 raw materials based on vegetables and animal sources, were tested (meals from: beans, camelina, soya, peas, sunflower, wheat, krill, degreased krill, chicken, squid and fish). Animal proteins were selected as the most adequate proteins for this specie. In addition, it was demonstrated that vegetable proteins alone did not present a good nutritional balance; however, they could be used as an ingredient for *S. dumerili* diets in combination with other protein sources.

Based on the results obtained, fish meal was replaced for a mixture of animal and vegetable protein sources. This study was started with 39 g of fish and it was finished with 365 g after 154 days. Four levels of fish meal substitution were studied (0%, 33%, 66%, 100%). With the results obtained in this experiment, it concludes that the total substitution of fish meal was not viable due to the lower digestibility capacity of essential amino acids and energy. In addition, higher mortality level was found after the ingestion of this diet. It was concluded that a replacement of 66 % led to a successful growth, nutritional efficiency and better survival results.

Considering results obtained in the first protein source test, a second study was carried out. It started with 530 g of fish (the fish were feed with the same diets during all the growth period) and ended up with 850 g after 84 days. The three diets with the best results of the previous experiments were asayed (0%, 33%, 66% of fish meal substitution) with the aim to study their effect in the meat quality (fatty acids profile, heavy metals, sensory and organoleptic analyses) in fish with commercial size. At the end of the experiment a reduction of most fatty acids with the increase of substitution level was detected. In addition, heavy metal levels in muscle did not exceed in any case the values permitted by the European Commission. Thus, they do not present a risk for human health. Otherwise, some of the physicochemical properties of the *Seriola dumerili* fillet were affected, however, this effect was not reflected on the organoleptic analyses, where tasters were not able to appreciate differences between diets, excepting for the final color.

In the next experiment, the effect of replacing the fish oil for a mixture of vegetable oils (palm oil and linseed oil in a proportion of 4:1) was elucidated. This study was started with fishes that weighted 39 g and finished with 390 g after 154 days. Three substitution levels were used (0%, 50% and 100% of fish oil). It was shown that the vegetable mixture can be used to completely replace the fish oil for young *Seriola dumerili* fishes, without affecting growth and nutrient efficiency. In addition, fatty acids profile in diet, fulfilled the need of *Seriola dumerili* juveniles without influencing their health and their final nutritional characteristics.

Based on the results obtained in this Ph. D thesis, it can be confirmed that feeding *Seriola dumerili* with fish meal substitution during long periods of time, do not affects negatively its growth and quality parameters such as fatty acids profile, especially with substitutions under than 66%. Good results were also obtained with lower percentages of substitution levels.

Regarding to the lipid substitution, a complete substitution of fish oil for vegetable oils was feasible for young *Seriola dumerili*, without

affecting their growth, yield, feed utilization and health, which should be considered in future feed formulations specific to this species.

Abreviaturas

Castellano

AAE: Aminoácidos esenciales
ARA: Ácido araquidónico
DHA: Ácido docohexanoico
EB: Energía bruta
EPA: Ácido eicosapentanoico
GB: Grasa bruta
HUFA: Ácidos grasos de cadena larga o altamente insaturados
IBE: Índice de beneficio económico
IBE_{st}: Índice de beneficio económico estándar
ICA: Índice de conversión del alimento
ICE: Índice de conversión económico
PB: Proteína bruta
PUFA: Ácidos grasos poliinsaturados
PV: Precio de venta del pescado en el mercado
RA: Rendimiento del aceite de pescado
RH: Rendimiento de la harina de pescado
SEM: Error estándar de la media
TCI: Tasa de crecimiento instantáneo
IT: Índice trombogénico
IA: Índice aterogénico
FLQ: Índice de calidad de los lípidos

English

AA: Aminoacids
AAR: Amino acid ratio
AARE: Amino acid retention efficiency
ADC: Apparent digestibility coefficient
AIA: Acid insoluble ash
AP: Animal proteins
ARA: Arachidonic acid
CF: Condicion factor
CP: Crude protein
CS: Chemical score
DAARE: Digestible amino acid retention efficiency
DEI: Digestible energy intake

DHA: Docosahexaenoic acid
DMA: Dimethyl acetals
DP: Digestible protein
DPI: Digestible protein intake
EAA: Esencial aminoacids
EFA: Essential fatty acids
EPA: Eicosapentaenoic acid
FCR: Feed conversion ratio
FI: Feed intake
FLQ: Flesh lipid quality
FM: Fish meal
FO: Fish oil
HSI: Hepatosomatic index
HUFA: Highly unsaturated fatty acids
IA: Index of atherogenicity
IPR: Ingested protein retention
IT: Index of trombogenicity
LA: Linoleic acid
LC-PUFA: Long chain poly unsaturated fatty acids
LNA: Linolenic acid
LO: Linseed oil
MF: Mesenteric fat index
MUFA: Mono-unsaturated fatty acids
OI: Oser's index
PER: Protein efficiency ratio
PNEP: Percentage of non-edible portion
PO: Palm oil
PUFA: Poly unsaturated fatty acids
SFA: Saturated fatty acids
SGR: Specific growth rate
VO: Vegetable oil
VSI: Viscerosomatic index
WW: Wet weight

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Capítulo 1
INTRODUCCIÓN GENERAL

1. Estado actual de la acuicultura

El aumento del suministro mundial de pescado para consumo humano ha superado al crecimiento de la población en los últimos 50 años, creciendo a un ritmo del 3,2% anual, dando lugar a un incremento de la disponibilidad *per cápita*. El consumo de pescado *per cápita* se ha visto incrementado de 16,1 kg en 2001 hasta superar los 20 kg en 2014 (FAO, 2018). Con la creciente demanda de pescado y la producción actual de los caladeros de pesca, que se encuentran estabilizados por debajo de los 100 millones de toneladas por año desde los años 90 (Figura 1.1), se hace necesario buscar otras fuentes de obtención de pescado que no comprometan los recursos naturales, como es el caso de la acuicultura.

La FAO describe la acuicultura como el cultivo de organismos acuáticos tanto en zonas costeras como del interior, implicando la intervención del ser humano en el proceso para aumentar la producción.

La acuicultura es el sector de producción de alimentos con mayor crecimiento, representando casi el 50% del pescado destinado a la alimentación a nivel mundial. Su actividad es llevada a cabo desde los países más pobres hasta las grandes multinacionales, siendo producidas en la actualidad cerca de 580 especies de animales y plantas acuáticas.

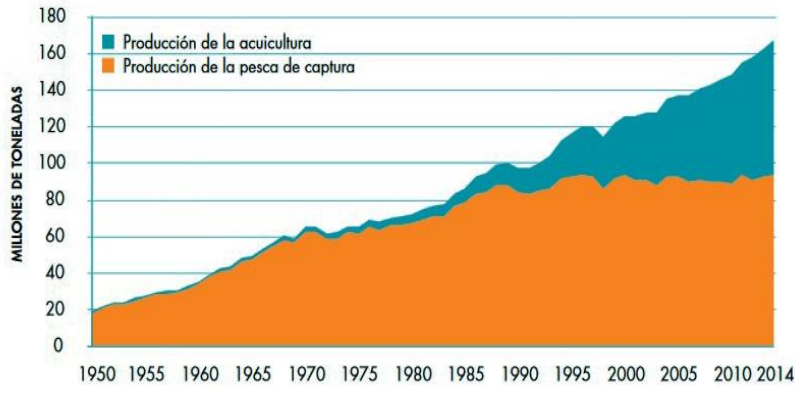

Figura 1.1 Producción mundial de la pesca y la acuicultura. FAO (2018)

La producción acuícola mundial se ha duplicado en los últimos años, pasando de los 32,4 millones de toneladas en el año 2000 a los 66 millones de toneladas en el año 2012, debiéndose fundamentalmente a la producción continental en países como China.

En el año 2016, la producción mundial de pescado alcanzó los 170,9 millones de toneladas (Tabla 1.1), de los cuales un 47% procede de la acuicultura (80 millones de toneladas). De estos 170 millones de toneladas de la producción total, alrededor del 88% (más de 151 millones de toneladas) fueron destinados al consumo humano directo, un porcentaje que ha ido en aumento significativamente en los últimos años. De este 88%, el 44% se comercializó como vivo, fresco o refrigerado, siendo el favorito para los consumidores y el que presenta un precio más elevado, mientras que un 31% que fue comercializado como pescado congelado.

El 12% no empleado para consumo humano (unos 20 millones de toneladas), fue destinado a la producción de harinas y aceites de pescado.

Tabla 1.1 Producción mundial de la pesca y la acuicultura (millones de toneladas)[a]. (FAO, 2018).

	2011	2012	2013	2014	2015	2016
Producción						
Pesca captura						
Continental	10,7	11,2	11,2	11,3	11,4	11,6
Marina	81,5	78,4	79,4	79,9	81,2	79,3
Pesca de captura total	**92,2**	**89,6**	**90,6**	**91,2**	**92,7**	**90,9**
Acuicultura						
Continental	38,6	42,0	44,8	46,9	48,6	51,4
Marina	23,2	24,4	25,4	26,8	27,5	28,7
Acuicultura total	**61,8**	**66,4**	**70,2**	**73,7**	**76,1**	**80,0**
Total a nivel mundial	**154,0**	**156,0**	**160,7**	**164,9**	**168,7**	**170,9**
Utilización						
Consumo humano	130,0	136,4	140,1	144,8	148,4	151,2
Uso no alimentario	24,0	19,6	20,6	20,0	20,	19,7
Población (miles de millones)	7,0	7,1	7,2	7,3	7,3	7,4
Consumo *per capita* (kg)	18,5	19,2	19,5	19,9	20,2	20,3

[a]excluidos los mamíferos acuáticos, cocodrilos, lagartos y caimanes, las algas y otras plantas acuáticas.

En Europa, en 2016, el pescado procedente de la acuicultura alcanzó los 2,9 millones de toneladas; destinándose prácticamente su totalidad al consumo humano,suponiendo una venta de casi 55 billones de euros, un 15% más que en 2015, presentando un crecimiento general en prácticamente todos los países miembros de la Unión Europea, siendo España el país líder alcanzando los 10,5 billones de euros.

En España el consumo *per cápita* de pescado es de 45,2 kg, siendo superado únicamente por Portugal. Este consumo supone un gasto de unos 227 euros al año.

El aumento de la ingesta de pescado ha provocado una alimentación mas variada y nutritiva, mejorando las dietas, en las que el pescado representa alrededor del 17% de la ingesta proteica animal de la población mundial y el 6,7% de las proteínas totales, siendo este una

fuente de proteínas de alta calidad, fácil digestión y que contiene todos los aminoácidos esenciales y además aporta grasas esenciales como los ácidos grasos *n*3 (EPA y DHA), vitaminas A y D, así como vitaminas del grupo B y minerales como el yodo, selenio, zinc, hierro, calcio, fósforo y potasio (EUMOFA, 2017; FAO, 2018).

2. Retos de la acuicultura mediterránea

Históricamente, la actividad acuícola en España se ha desarrollado concentrándose en un número limitado de especies; los peces marinos mayormente producidos son dorada (*Sparus aurata*), lubina (*Dicentrachux labrax*), rodaballo (*Scophthalmus maxima*), corvina (*Argyrosomus regius*) y lenguado (*Solea senegalensis*). La producción de estas especies ha ido aumentando considerablemente desde sus inicios en los años 80 hasta 2009 (Figura 1.2); sin embargo, desde entonces ha sufrido un estancamiento que en 2015 comenzó a superarse ligeramente (APROMAR, 2019).

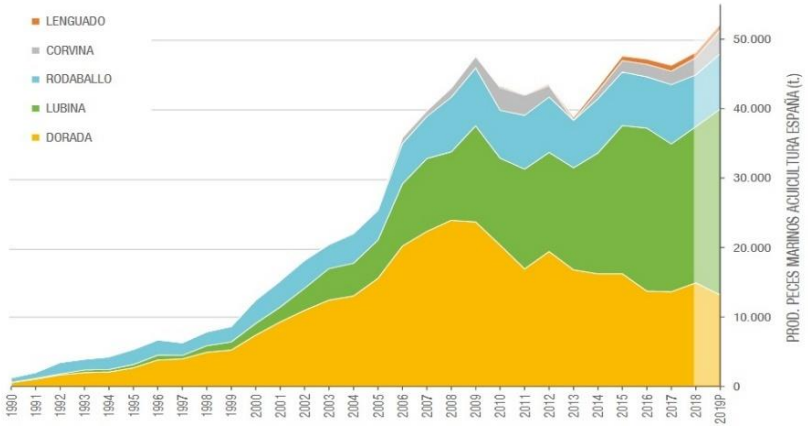

Figura 1.2 Producción de las principales especies marinas de acuicultura (t) en España en el periodo 1990-2019 APROMAR (2019).

Este estancamiento se ha debido principalmente a un exceso de producción, junto con el aumento de importaciones de pescado de acuicultura procedente de otros países como Grecia, y una reducción en la tasa de consumo por parte de la población.

Desde el año 2010, el consumo de productos marinos en los hogares españoles ha disminuido, siendo las principales especies consumidas merluza, sardina, salmón, lenguado, bacalao y atún (APROMAR, 2019). También se ha reducido el consumo en España de pescado fresco, que representa el 40% del total en los hogares, seguido de las conservas y productos congelados, por lo que una ampliación de la oferta disponible a los consumidores, atractiva y de calidad, podría reactivar este consumo.

Estos factores han derivado en una disminución del precio de venta, como es el caso de la lubina, que ha descendido de 5,50 €/kg a 4,60 €/kg o la dorada, que ha pasado de 4,80 €/kg a 3,60 €/kg (aproximadamente en el periodo 2016-2018, precios de salida en mercados mayoristas) (APROMAR, 2019). Este descenso ha producido una disminución considerable de la rentabilidad de la producción, debido a que los costes de producción se mantienen estables mientras el beneficio es mucho menor.

Para mejorar este escenario, un buen punto de partida es la producción de nuevas especies que amplíen la oferta al consumidor, utilizando las instalaciones ya disponibles, siempre que estas especies presenten una serie de requisitos que puedan asegurar su viabilidad comercial (buena adaptación a la cautividad, crecimiento rápido en producción intensiva, conocimiento de sus requisitos biológicos y zootécnicos, así como un alto precio y una alta demanda en el mercado). Esta ampliación de las líneas de producción fortalecerá el sector y potenciará el crecimiento de la acuicultura mediterránea en el mercado internacional.

Una de las especies que aparecen como mejor opción para esta diversificación del mercado mediterráneo es la seriola mediterránea (*Seriola dumerili)*, considerada como una de las especies con mayor proyección debido a su rápido crecimiento (Figura 1.3), hasta 10 veces superior que otras especies mediterráneas como la dorada o la lubina,

pudiendo llegar casi a los 3 kg en un año (Asche & Bjondal, 2011). Así mismo, tiene una buena capacidad de adaptación a las condiciones de producción y tolera bastante bien las altas densidades.

Los primeros estudios llevados a cabo con seriola en cautividad (de la Gándara, 2006; Jover et al., 1999; Mazzola et al., 2000) alimentada con piensos extrusionados, ya demostraron que esta especie tiene una tasa de crecimiento muy superior a las especies ya consolidadas en acuicultura.

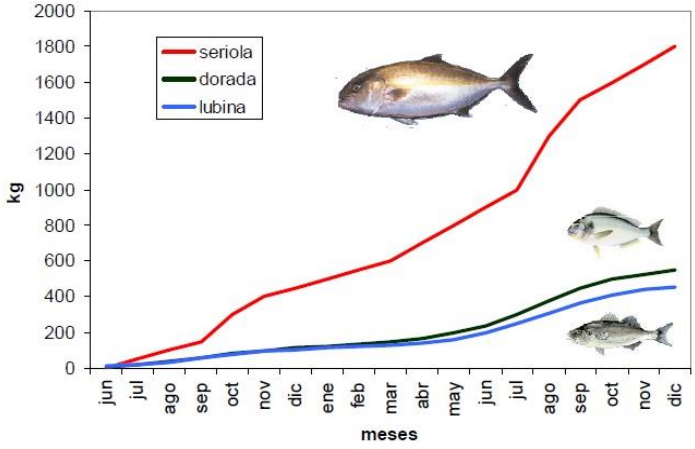

Figura 1.3 Curvas comparativas de crecimiento de la seriola, dorada y lubina, criadas en instalaciones experimentales en tierra (de la Gándara, 2006).

Por otra parte, su carne tiene una excelente calidad y presenta un alto valor comercial (de entre 10-20 €/kg en el mercado mediterráneo y que ha alcanzado hasta los 30 €/kg en Japón) (Abellán-Martínez & Arnal, 2013) siendo además altamente apreciada en el mercado a lo largo del mundo (Nakada, 2000). Todo esto hace que tenga las cualidades ideales como especie prometedora en acuicultura (Marino et al., 1995).

Biológicamente es muy similar a la *Seriola quinqueradiata*, en cuanto a morfología y comportamiento, cuya producción está estabilizada en el mercado japonés y es ampliamente producida y controlada con unas 150.000 toneladas al año.

3. Aspectos biológicos de la *Seriola dumerili*

La *Seriola dumerili* es un teleósteo marino, perteneciente a la familia Carangidae, al cual pertenecen 30 géneros que incluyen unas 150 especies. Es la nativa del Mar Mediterráneo y la especie más grande del género (Poortenaar et al., 2001).

Es un pez de cuerpo alargado, fusiforme y aplanado lateralmente, con el dorso más prominente que el vientre (Figura 1.4). Presenta un color gris azulado u oliváceo en su parte dorsal y plateado en la parte ventral, con una franja lateral dorada en los ejemplares adultos. Tiene dos aletas dorsales, la primera más baja que la segunda, y tanto la segunda aleta dorsal como la aleta anal, tienen el primer radio más largo. Las aletas pectorales son más cortas que las ventrales, y tiene una aleta caudal ahorquillada y muy potente, con el pedúnculo estrecho. Llegan a alcanzar los 190 cm y los 80 kg de peso.

Figura 1.4 Ejemplar de seriola mediterránea (*Seriola dumerili*).

Su distribución es muy amplia, encontrándose en el océano Índico, en el Pacífico occidental y en el Atlántico, de Canadá a Brasil, así como en el mar Mediterráneo. Es propia de aguas subtropicales (Figura 1.5).

Figura 1.5 Distribución global de la seriola mediterránea (*Seriola dumerili*). Fuente: www.fishbase.org

Presenta hábitos pelágicos o epibentónicos, pueden encontrarse en solitario o formando grupos, son depredadores oportunistas de gran velocidad, alimentándose principalmente de crustáceos, cefalópodos y peces, variando su dieta en función del tamaño (Andaloro & Pipitone, 1997).

Especie gonocórica, con un desarrollo ovárico síncrono por grupos. alcanzan la madurez en torno a los 4-5 años, con un periodo de desove que varía dependiendo de las zonas de distribución: en el Atlántico se extiende entre marzo y mayo, de mayo a julio en el Mediterráneo, y entre febrero y julio en el pacífico, desovando siempre en zonas cercanas a la costa.

4. Situación actual de la *Seriola dumerili* en acuicultura

Las especies del género *Seriola* son principalmente producidas en Asia, siendo la seriola japonesa (*Seriola quinqueradiata)* la más representativa, cuya producción en cautividad viene desarrollándose desde los años 60 del pasado siglo y que destaca ampliamente con una producción mundial de 140.000–160.000 toneladas al año siendo los productores mayoritarios Japón y China (FAO, 2018; Lovatelli et al., 2013).

La seriola australiana (*Seriola lalandi)* ha tenido un gran desarrollo en los últimos años en países como Australia, Japón o Sudáfrica entre otros, siendo Australia el mayor productor de esta especie con unas 5000 toneladas al año, seguido de Japón con 4500 toneladas en 2013 (Miller et al., 2011).

La *Seriola rivoliana,* se la ha señalado como especie potencial en la zona de Sudáfrica (O'Neill et al., 2015) y en la actualidad se producen en torno a 500 toneladas en el sur de EEUU y Hawai (Sims, 2013).

Por último, la *Seriola dumerili*, es la segunda especie con una mayor historia en la producción del género, que comenzó en Japón en 1978 y actualmente es considerada de gran importancia, y representa el 30% de la producción de Japón (unas 39.000 toneladas) (FAO, 2018). En la década de los 80 comenzó a producirse en el Mediterráneo, siendo los principales productores, España (Cádiz, Islas Baleares, Murcia y Tarragona), Italia y Malta (Sicuro & Luzzana, 2016).

En 2016, el 78% de la seriola consumida procedía de la pesca extractiva y el 22% restante de la acuicultura. Del porcentaje de seriola producida en cautividad, el 92% se realiza dentro del continente asiático (74% en Japón, 11% en China y 7% en Korea) (FAO, 2018).

Sin embargo, a pesar de los aspectos positivos, la adaptación a la cautividad de una especie pelágica predadora como ésta no es sencilla, y ha presentado diferentes problemas en los que se comenzó a trabajar, hace ya dos décadas, y que han limitado su desarrollo comercial. Los dos grandes problemas que ha presentado han sido las dificultades para su reproducción en cautividad y los problemas relacionados con patologías asociadas a su producción.

La problematica relacionada con la patología ha estado asociada principalmente a distintos parásitos como *Paradeontacylix* sp., *Zeuxapta seriolae* o *Cryptocaryon irritans*. En estudios previos en *Seriola quinqueradiata* se ha visto como la sustitución de harina de pescado por fuentes proteicas alternativas afecta de forma significativa a los peces, produciendo altas mortalidades y aumenta su susceptibilidad a patógenos (Maita et al., 2006).

5. La nutrición de la *Seriola dumerili*

El objetivo principal al criar una especie en cautividad, es desarrolar un pienso que cubra sus necesidades nutritivas, por ello hay que estudiar los niveles óptimos de nutrientes en el pienso (especialmente proteínas y lípidos), lo que ha sido estudiado en los trabajos iniciales que existen sobre esta especie (Jover et al., 1999; Tomás-Vidal et al., 2008).

Una vez conocidas las necesidades nutricionales de la especie, el siguiente objetivo es mejorar y abaratar los piensos, sustituyendo la harina y el aceite de pescado por otras fuentes alternativas de menor coste y que sean capaces de suplir las necesidades de la especie (en aminoácidos y ácidos grasos) (Tomás-Vidal, 2003).

5.1. Necesidades proteicas y lipídicas de *S. dumerili*

Las especies del género seriola tienen unos requerimientos proteicos elevados (450-550 g/kg) para obtener el máximo crecimiento, como se ha visto es estudios previos en *Seriola dumerili* (Jover et al., 1999; Takakuwa et al., 2006b; Talbot et al., 2000; Tomás-Vidal et al., 2008) en los que se obtuvieron los siguientes resultados (Tabla 1.2).

Tabla 1.2 Resultados de crecimiento de la seriola mediterránea obtenidos por diferentes autores según los distintos niveles de proteína y lípidos.

	% PB/GB	TCI	ICA	Ingesta	Días	Pi Peces
Jover et al., 1999	**50/14**	**0,72**	**2,63**	**1,33**	307	150 g
	50/17	0,67	2,44	1,27		
	45/14	0,64	2,88	1,38		
	45/17	0,65	2,95	1,41		
Talbot et al., 2000	42/18	1,12			118	100 g
	42/22	1,12				
	42/25	1,14				
	42/30	1,12				
Takakuwa et al., 2006b	**42/13**	**2,60**		**3,43**	40	70 g
	42/18	2,69		3,41		
	42/23	2,51		3,54		
	47/13	2,96		3,30		
	47/18	2,82		3,31		
	47/23	2,69		3,42		
	53/13	3,04		3,22		
	53/18	2,76		3,46		
	53/23	2,95		3,07		
Tomás-Vidal et al., 2008	40/26	0,31	6,7	1,68	152	490 g
	45/26	0,37	6,1	1,75		
	50/26	0,46	5,4	1,65		
	50/18	0,46	4,0	1,57		
	55/18	**0,48**	**4,2**	**1,56**		

Donde PB/GB: proteína bruta/grasa bruta; TCI: tasa de crecimiento instantáneo; ICA: Índice de conversión del alimento; Pi: peso inicial. En negrita los mejores resultados obtenidos en cada experimento.

Jover et al. (1999) probaron distintas proporciones de proteínas/lípidos para observar cual era la proporción óptima para obtener el mayor crecimiento posible en *S. dumerili* y observaron que los peces crecieron mejor con un nivel de lípidos del 14% comparado con el de 17% y con un nivel de proteína del 50%.

Talbot et al. (2000), observaron que con niveles bajos de proteína (40%) y elevada energía (30% lípidos y 17,3 g/MJ PB/EB) tenían crecimientos similares en *S. dumerili* que con baja energía (18% lípidos y 18,7 g/MJ PB/EB)

Takakuwa et al. (2006b) realizaron un estudio con juveniles de *S. dumerili* en los que se probaron tres niveles de proteína (42, 47 y 53%) y tres de lípidos (13, 18 y 23%), vieron que el mejor crecimiento se

obtenía con la dieta que presentaba un 42% de proteína y un 13% de lípidos.

Tomás-Vidal et al. (2008) estudiaron el efecto del ratio proteína digestible/energía digestible en el crecimiento de la en *S. dumerili* y comprobaron que la proporción para un óptimo crecimiento estaba en 55% de proteína y 18% de lípidos.

5.2. Fuentes proteicas en piensos de *Seriola dumerili*

La fuente proteica tradicional en los piensos de acuicultura es la harina de pescado, debido a su alto contenido proteico (superior al 60% de proteína bruta (PB), a su alta digestibilidad y buen equilibrio aminoacídico, así como por su aporte de ácidos grasos esenciales, alto contenido en vitaminas y minerales, lo que la convierten en la fuente perfecta para los piensos para especies piscícolas carnívoras.

El uso de la harina de pescado en la actualidad se ha visto limitado desde hace unos años debido a la sobreexplotación, su precio se ha elevado, y su disponibilidad ha disminuido, lo que obligó al sector a buscar otras opciones para tener una menor dependencia de la misma.

Las fuentes proteicas alternativas incluyen un amplio rango de proteínas tanto de origen animal como vegetal que pueden ser usadas en los piensos de acuicultura con precios muy competitivos (Martínez-Llorens et al., 2012). A pesar de esta ventaja, los resultados que se han obtenido en estudios previos en seriola, indican que una sola fuente proteica no puede sustituir a la harina de pescado totalmente, ni siquiera a altos niveles, como se observó en Tomás et al. (2005) en el que sustituyó la harina de pescado por turtó de soja, donde una sustitución superior al 30% produjo un peor crecimiento y una baja eficiencia nutritiva.

Takakuwa et al. (2006a) probaron la sustitución de la harina de pescado por harina de pollo, y observaron que los peces mostraron un crecimiento adecuado hasta un 40% de sustitución sin suplementación de aminoácidos, pero añadiendo cierta proporción de éstos, la sustitución podía elevarse hasta el 60%.

Dawood et al. (2015) utilizaron dietas con distintos niveles de inclusión de soja y observaron que a partir del 45% de sustitución, el crecimiento y la digestibilidad comenzaban a disminuir.

La calidad de la proteína viene dada por su perfil aminoacídico y la disponibilidad de estos aminoácidos. El perfil ideal de aminoácidos podría ser obtenido usando una combinación adecuada de proteínas, ya que las funciones metabólicas requieren de unos aminoácidos concretos, y la formación de la proteína dependerá de la ingestión de sus componentes estructurales, por lo que es crucial para los peces el ingerir y asimilar de forma correcta los aminoácidos adecuados a sus necesidades los cuales procederán de estas fuentes proteicas.

Los aminoácidos deben estar equilibrados, aproximándose al perfil ideal de aminoácidos del músculo de los peces. Las necesidades de aminoácidos esenciales (AAE) y los aminoácidos del músculo presentan un alto grado de correlación (Oliva-Teles, 2012).

Por tanto, si se consigue un buen perfil aminoacídico a base de la mezcla de diferentes ingredientes proteicos, se podría realizar una buena sustitución de la harina de pescado por fuentes proteicas alternativas, obteniendo un mejor crecimiento y una mejor retención de nitrógeno, como ocurre en otras especies como el salmón (Carter & Hauler, 2000; Espe et al., 2006) o la dorada (Kissil & Lupatsch, 2004; Monge-Ortiz et al., 2016; Sánchez-Lozano et al., 2009, 2011), y una reducción de los antinutrientes presentes en el pienso evitando así la necesidad de suplementar con aminoácidos sintéticos (Oliva-Teles, 2012).

Sin embargo, la alta sustitución de la harina de pescado por fuentes proteicas alternativas puede producir efectos no deseados en los peces, causados principalmente por los antinutrientes de las fuentes vegetales (Francis et al., 2001) que pueden causar una baja actividad enzimática, baja digestibilidad proteica (Spinelli et al., 1983) y aumentar la susceptibilidad de los peces a patógenos, causar inmunospresión y altas mortalidades (Estruch et al., 2015; Sitjà-Bobadilla et al., 2005).

5.3. Fuentes lipídicas en piensos de *Seriola dumerili*

La necesidad de sustituir el aceite de pescado por fuentes lipídicas alternativas es cada vez más importante en la acuicultura, debido, al igual que ocurre con las harinas de pescado, a un aumento de precios y a la disminución de la disponibilidad, lo que ha obligado a la industria a buscar fuentes lipídicas alternativas.

Los aceites vegetales tienen una especial relevancia como sustitutos del aceite de pescado, debido a su menor coste, a su baja concentración de dioxinas y otros contaminantes orgánicos y a sus adecuados niveles de producción (Sales & Glencross, 2011). La producción de estos aceites ha ido en aumento en los últimos años, contrariamente a lo que ha ocurrido con el aceite de pescado, siendo los aceites de palma, soja, colza y girasol los más abundantes, y además presentan un precio menor al del aceite de pescado.

Otra ventaja que presentan los aceites vegetales es su buena utilización por parte de los peces, que los metabolizan y los utilizan como fuente de energía (Bell et al., 2001; Regost et al., 2003), sin embargo, el principal problema que presentan es su composición en ácidos grasos, ya que son pobres en PUFA y *n-3* y ricos en *n-6* y *n-9* lo que limita su uso como única fuente proteica para la alimentación de peces.

Para la mayor parte de organismos marinos, los ácidos grasos de cadena larga o ácidos grasos altamente insaturados (HUFAs), son ácidos grasos esenciales, ya que los peces marinos no poseen los mecanismos necesarios para su síntesis, por lo que deben estar presentes en su dieta (Fountoulaki et al., 2009). Son concretamente el EPA (ácido eicosapentanoico, 20:5 *n*-3), el DHA (ácido docosahexanoico, 22:6 *n*-3) y el ARA (ácido araquidónico, 20:4 *n*-6). Su presencia en el alimento es fundamental no solo para un correcto crecimiento de la especie, sino también para su salud y una adecuada calidad de la carne en los peces (Bell et al., 2003).

La utilización de fuentes alternativas al aceite de pescado está condicionada por la necesidad de mantener unos niveles de EPA y DHA en el producto final destinado al consumo humano, ya que los

alimentos de origen marino son la fuente más importante de estos HUFAs en las dietas humanas (Hunter & Roberts, 2000) Así pues, la sustitución total de aceites de pescado como ingrediente o como componente de las harinas de pescado no es teóricamente factible en piensos para peces marinos.

Hasta la fecha se han realizado numerosos estudios de sustitución del aceite de pescado por fuentes lipídicas alternativas en multitud de especies como la dorada (Benedito-Palos et al., 2007, 2008; Fountoulaki et al., 2009), lubina (Izquierdo et al., 2003; Mourente & Bell, 2005), pargo rojo, (Huang et al., 2007) lenguado (Regost et al., 2003), salmón (Ruyter et al., 2006; Torstensen et al., 2000) o cobia (Trushenski et al., 2011). En estos estudios, el crecimiento se ha visto poco o nada afectado, siempre que el nivel mínimo de ácidos grasos esenciales esté cubierto. Sin embargo, los peces alimentados con aceites vegetales presentan importantes modificaciones en los ácidos grasos de sus tejidos, entre los que encontramos altos niveles de C18 PUFA y aparecen reducidos los niveles de *n*-3 HUFA, especialmente EPA y DHA (Bell et al., 2001; Bell & Sargent, 2003; Martins et al., 2012).

Actualmente existe un escaso conocimiento de las necesidades de ácidos grasos esenciales, así cómo del efecto de la sustitución de aceites de pescado en piensos para *Seriola dumerili*.

6. Calidad del filete de pescado en función de la alimentación

Existen muchos factores que pueden afectar a la calidad de la carne, pero el más importante es la alimentación durante el periodo de crecimiento. Esta influirá en la composición del filete (lípidos y perfil de ácidos grasos), en la bioseguridad (ausencia de bacterias, parásitos o componentes químicos) y en sus propiedades organolépticas.

En cuanto a la composición del filete, la sustitución de la harina de pescado puede afectar al crecimiento, pero la composición aminoacídica final que presentará el filete está determinada genéticamente y no se ve afectada por el tamaño o la dieta (Kaushik, 1998), como se ha podido ver en diferentes especies como la dorada

(Sánchez-Lozano et al., 2009), el salmón (Refstie et al., 2004) o la seriola (Tomás et al., 2005). Sin embargo, el factor que sí puede influir en el sabor de la carne es la presencia de aminoácidos libres como consecuencia de una mala combinación de los ingredientes proteicos o de aminoácidos sintéticos en la fabricación de los piensos (Kasumyan, 2016).

Como se ha comentado anteriormente, el nivel de lípidos y la composición en ácidos grasos del pienso sí afectan a la composición final del filete del pez. En estudios previos con especies como el pargo o la dorada se ha visto que una reducción de la concentración lipídica o una reducción en el porcentaje de ácidos grasos esenciales reduce la jugosidad de las muestras (García-Romero et al., 2014; Izquierdo et al., 2003; 2005).

En cuanto a la bioseguridad del filete (ausencia de bacterias, parásitos o componentes químicos), uno de los aspectos más importantes es la presencia de metales pesados. Los metales pesados son esenciales para el hombre a bajas concentraciones, pero pueden resultar tóxicos si las concentraciones son muy elevadas, y llegar a producir una situación de peligro para la salud (Oehlenschleäger, 2002). Los metales que nomalmente se encuentran asociados a un peligro potencial para el ser humano son arsénico, cadmio, cromo, plomo y mercurio. Es importante conocer la concentración de dichos metales pesados en el filete para evaluar los posibles riesgos asociados a ellos para el ser humano (Pérez Cid et al., 2001).

Los metales pesados pueden detectarse en diferentes órganos del pez (Eboh et al., 2006). Aquellos procedentes del medio natural se acumulan en las branquias, mientras que los metales pesados que son ingeridos con el alimento, se acumulan en el tracto digestivo y el hígado (Bahnasawy, 2009; El-Moselhy et al., 2014; Yousafzai et al., 2012), por lo que el músculo es un área de menor acumulación y por tanto los metales no llegarían al consumidor.

Por último, los ingredientes con los que se fabrican los piensos pueden afectar a la frescura y a las propiedades sensoriales del filete, que pueden resumirse en color, sabor, textura, estructura, estabilidad, olor, apariencia y aceptabilidad (Álvarez et al., 2008; Izquierdo et al.,

2005; Regost et al., 2003; Torstensen et al., 2005; Turchini et al., 2007). Estudios sobre esto han sido llevados a cabo previamente en otras especies como la trucha (*Oncorrhynchus mykiss*), donde De Francesco et al. (2004) probaron diferentes niveles de sustitución de harinas de pescado por proteínas vegetales y encontraron diferencias en los análisis organolépticos y en el color. En dorada, también se estudió el efecto de la sustitución de la harina de pescado por fuentes vegetales (De Francesco et al., 2007), produciendo un efecto mínimo en la calidad del pescado. En salmón (*Salmo salar*), Bjerkeng et al. (1997), probaron diferentes niveles de sustitución de harina de pescado por harina de soja sin que se vieran afectados los parámetros de calidad, al igual que Valente et al. (2011) en lenguado (*Solea senegalensis*), estudiandodiferentes sustituciones de harinas de pescado por mezclas vegetales, sin diferencias significativas.

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Capítulo 2
JUSTIFICACIÓN Y OBJETIVOS

Actualmente el sector acuícola se enfrenta al reto de expandir y desarrollar la oferta de nuevos productos para el mercado, así como la introducción de nuevas especies, para aumentar la rentabilidad de la producción. Entre estas especies, se encuentra como candidata la seriola mediterránea (*Seriola dumerili)*, por su elevado crecimiento y su alta calidad de la carne.

Para producir la seriola mediterránea a nivel comercial es necesario el desarrollo de piensos que permitan obtener de manera eficaz un rápido crecimiento, una óptima eficiencia del alimento con bajas mortalidades, así como una elevada calidad de la carne de cara al consumidor. Por otro lado, y debido a la limitación, tanto económica como medioambiental, del uso de harinas y aceites de pescado para la alimentación de peces, es fundamental la inclusión de materias primas alternativas para formular piensos específicos de manera que no afecten negativamente a su productividad. Los estudios llevados a cabo sobre la sustitución de las harinas y aceites de pescado por fuentes proteicas y lipídicas alternativas en piensos para *Seriola dumerili* son muy limitados.

Es por ello, que el objetivo principal de este trabajo fue contribuir al diseño de un pienso adecuado a las necesidades nutritivas de esta especie con los mínimos niveles de harinas y aceites de pescado sin perjudicar al crecimiento, aprovechamiento nutritivo y calidad del filete.

Para llevar a cabo este objetivo, se plantearon los siguientes objetivos específicos:

- Evaluar la digestibilidad y la calidad de la proteína de una serie de ingredientes seleccionados para la alimentación de *S. dumerili.*

- Evaluar el efecto de la sustitución de la harina de pescado por fuentes proteicas alternativas animales y vegetales en piensos para *S. dumerili* sobre:
    - El crecimiento, la eficiencia nutritiva y la composición corporal.
    - La digestibilidad de las dietas utilizadas.

  - El aprovechamiento nutritivo y la eficiencia de retención de los aminoácidos.
  - Los niveles de metales pesados en el filete.
  - El perfil de ácidos grasos en el filete.
  - La calidad organoléptica y sensorial de los filetes.

- Evaluar la sustitución de aceite de pescado por aceites vegetales sobre:
  - El crecimiento y la eficiencia alimenticia.
  - La salud de los peces.
  - La composición nutricional del pescado y calidad lipídica.

Capítulo 3
RESUMEN DE LOS EXPERIMENTOS

Resumen de los Experimentos

La presente tesis doctoral fue dividida en 4 diferentes experimentos como puede apreciarse en la figura 3.1.

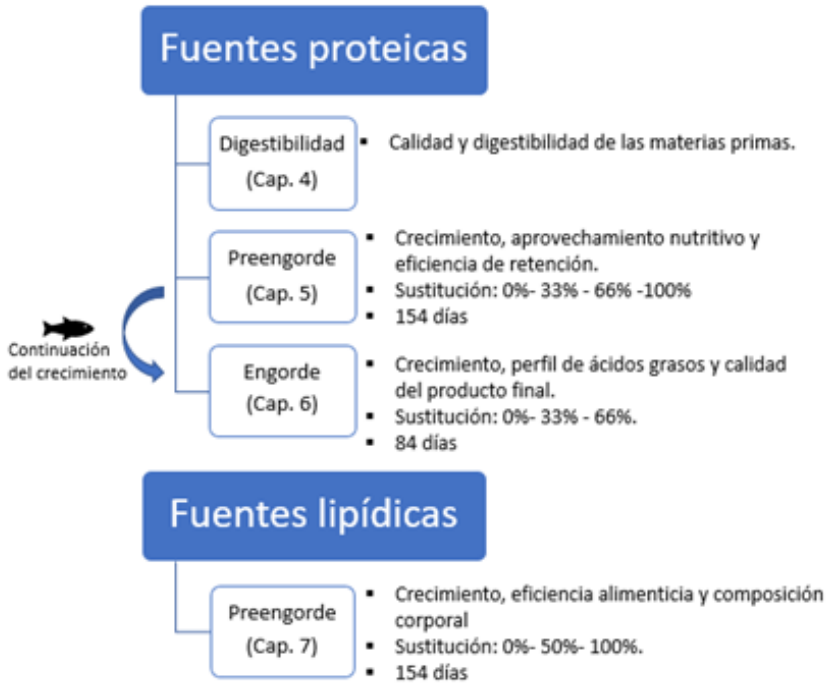

Figura 3.1 Resumen de los experimentos realizados durante la presente tesis doctoral.

Los trabajos han sido financiados por los siguientes proyectos:

- "Sustitucion de harina y aceite de pescado en dietas para *Seriola dumerili*. Pruebas de alimentacion y estudios analiticos: proteínas". Financiado por el Ministerio de Economía y Empresa, Ref: AGL2011-30547-C03-02.
- "Ingredientes alternativos en piensos para *Seriola dumerili*: efecto en el crecimiento y en la calidad de la carne". Financiado por la Generalitat Valenciana, Ref: AICO/2015/123.

Se han realizado las siguientes publicaciones:

Tomás-Vidal, A., Monge-Ortiz, R., Jover-Cerdá, M., & Martínez-Llorens, S. (2019). Apparent digestibility and protein quality evaluation of selected feed ingredients in *Seriola dumerili*. *Journal of the World Aquaculture Society*, *50*(4), 842-855.

Monge-Ortiz, R., Tomás-Vidal, A., Rodriguez-Barreto, D., Martínez-Llorens, S., Pérez, J. A., Jover-Cerdá, M., & Lorenzo, A. (2018). Replacement of fish oil with vegetable oil blends in feeds for greater amberjack (*Seriola dumerili*) juveniles: Effect on growth performance, feed efficiency, tissue fatty acid composition and flesh nutritional value. *Aquaculture Nutrition*, *24*(1), 605-615.

Monge-Ortiz, R., Tomás-Vidal, A., Gallardo-Álvarez, F. J., Estruch, G., Godoy-Olmos, S., Jover-Cerdá, M., & Martínez-Llorens, S. (2018). Partial and total replacement of fishmeal by a blend of animal and plant proteins in diets for *Seriola dumerili*: Effects on performance and nutrient efficiency. *Aquaculture Nutrition*, *24*(4), 1163-1174.

Monge-Ortiz, R., Lemos-Neto, M.J., Falcó, S., Pagán, M.J., Godoy-Olmos, S., Jover, M., Tomás-Vidal, A. (2019) Growth, sensory and chemical characterization of Mediterranean yellowtail (*Seriola dumerili*) fed diets with partial replacement of fish meal by other protein sources. *Aquaculture Reports* (Under review).

Se han realizado las siguientes comunicaciones en congresos.

<u>Comunicaciones Orales:</u>

Navarro-Ramírez, A.I., Pagán, M.J., Gallardo, F.J., Lemos-Neto, M.J., Martínez-Juarez, L., Estruch, G., Godoy, S., Monge, R., Jover, M., Tomás-Vidal, A., Martínez-Llorens, S. (2015, October). Efecto de la sustitución de la harina y aceite de pescado sobre las características organolépticas del filete de seriola (crudo y cocinado) y las propiedades fisico-químicas, ópticas y texturales. XV Congreso Nacional de Acuicultura. Huelva, España.

Monge-Ortiz, R., Pagán, M.J., Vélez-Calabria, G., Estruch, G., Godoy-Olmos, S., Milián, M.C., Jauralde, I., Moñino, A., Martínez-Llorens, S., Jover-Cerdá, M., Tomás-Vidal, A. (2017, octubre) Caracterización química y sensorial de los filetes de *Seriola dumerili*, alimentados con dietas con sustitución parcial de harina de pescado por fuentes proteicas alternativas. XVI Congreso Nacional de Acuicultura. Zaragoza, España.

Comunicaciones formato póster:

Tomás-Vidal, A., Monge, R., Estruch, G., Godoy, S., Moñino, A.V., Jover-Cerdá, M. & Martínez-Llorens, S. (2014, October). Almost total replacement of fish meal by a mixture of plant and animal proteins in yellowtail diets (*Seriola dumerili*). XV Congreso Europeo de Acuicultura. San Sebastián, España.

Cruz-Castellón, C., Navarro-Ramírez, A.I., Estruch, G., Godoy, S., Monge, R.**,** Gallardo, F.J., Valero, M., Tomás-Vidal, A., Lemos, M., Martínez-Llorens, S. (2015, October). Efecto del aceite de pescado sobre el crecimiento, parámetros nutritivos y hematológicos de la *Seriola dumerili.* XV Congreso Nacional de Acuicultura. Huelva, España.

Lemos Neto, M.J., Monge-Ortiz, R., Martínez-Llorens, S., Falcó, S., Jover-Cerdá, M., Morgano, M.A., Tomás-Vidal, A. Niveis de metais pesados (As, Cd, Hg, Zn, Cu) em *Seriola dumerili* alimentadios com diferentes formulaçoes de raçoes. VII Simpósio de Controle de Qualidade do Pescado (SIMCOPE). Sao-Paulo, Brasil.

Capítulo 4
Apparent digestibility and protein quality evaluation of selected feed ingredients in *Seriola dumerili*.

Publicado en Journal of the World Aquaculture Society

A. Tomás-Vidal, R. Monge-Ortiz, M. Jover-Cerdá, S. Martínez Llorens (2019) Apparent digestibility and protein quality evaluation of selected feed ingredients in *Seriola dumerili*. Journal of the World Aquaculture Society, 50, 842-855.

ABSTRACT

The apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude lipid, and amino acids in fish, krill, squid, meat, defatted krill, soybean, wheat gluten, wheat, camilina, pea, sunflower, and fava bean meals were determined for juvenile *Seriola dumerili*. The results showed that the ADC of dry matter for yellowtail ranged from 57.7 to 87.2% for animal ingredients and from 42.2 to 82.2% for plant ingredients. An ADC of protein exceeding 90% was observed in fish meal, while camilina meal and fava bean meal presented the lowest values. Pea meal presented the lowest lipid ADC (83.5%). The availabilities were generally higher in animal ingredients than those in vegetal ones. Except camilina and fava bean meal, the other ingredients appear to be favorable for *Seriola dumerili* diets, especially the ones from animal sources. Lower case chemical score values (mínimum value from amino acid ratios [AARs]) were obtained in some vegetal ingredients (14–18%), while the highest ones were observed in marine ingredients (69–88%). According to Oser's Index, the most balanced protein for yellowtail with regard to essential amino acids was in krill, defatted krill, and fish meal (92–96%). So, animal sources are suitable as protein ingredients, but they could be enhanced through some essential amino acid supplementation.

1. INTRODUCTION

In the last two decades, the European aquaculture sector has shown an increasing interest and demand in fastgrowing species (Mazzola et al., 2000), such as Mediterranean yellowtail (*Seriola dumerili*), as they can improve the competitiveness of aquaculture companies.

The most expensive component in feed formulation in aquaculture is protein. This is especially important considering that Seriola species require high protein dietary levels (450, 550 g/kg) for maximum growth (Jover et al., 1999; Masumoto et al., 1998; Tomás-Vidal et al., 2008), and the dietary substitution of proteins with other nutrients—such as lipids or carbohydrates—is not the best solution for the formulation of feeds for *S. dumerili* (Jover et al., 1999). In this sense, fish meal is an ideal protein source for carnivorous fish because it contains a balanced amino acid composition, high content of phosphorous and minerals, and high digestibility coefficients. However, its high cost motivated its reduction in aqua feeds while maintaining the same protein levels from alternative protein sources.

Currently, in order to avoid the dependence on fish meal, aquaculture diets should contain high amounts of alternative proteins that are more widely available and that have lower and more stable market prices than this marine resource. Alternative proteins include a large range of plant or animal proteins with competitive prices (Martínez-Llorens et al., 2012). Despite these economic advantages, previous results on Mediterranean yellowtail show that alternative proteins cannot totally replace fish meal when they are used as the sole ingredient.

When soybean was included to replace over 30% of the fish meal, it resulted in poor growth and showed low nutrient efficiency (Tomás et al., 2005). Total fish meal substitution presents some limitations in fish diets, such as the antinutrients that are particularly contained in vegetable proteins (Krogdahl et al., 2010), causing a protease enzyme activity decrease and, therefore, low protein digestibility (Monge-Ortiz et al., 2016). Another issue related to the use of alternative protein sources is the inadequate profile of amino acids (Cerezo-Valverde et

al., 2013), which results in poor fish growth and protein deficiencies. This forces the use of supplemental diets with synthetic amino acids, which increases the feed price. The best growth and nitrogen retention occurs when a combination of protein sources is used, which include a combination of amino acids in order to complement one source with limiting ones, as is the case of *Sparus aurata* (Kissil & Lupatsch, 2004; Sánchez-Lozano et al., 2009), and evidence suggests that this is the case for other species as well, such as *Salmo salar* (Espe et al., 2006) or *S. dumerili* (Monge-Ortiz et al., 2018). For this reason, the combination of animal proteins (such as defatted krill and poultry meal) with plant proteins in fish diets could be beneficial to palliate the deficiencies in amino acids of some protein sources, thus reducing the antinutrient compounds and avoiding supplementation with synthetic amino acids (AAs), a lack of palatability (Oliva-Teles, 2012), and "green liver" (Takagi et al., 2005). The adequate proportion needed to simulate the ideal amino acid profile in diets for the Mediterranean yellowtail will require studies on the digestibility and the protein quality of these ingredients. Therefore, the evaluation of feed ingredients is crucial to nutritional research and feed development for aquaculture species (Glencross et al., 2007). The alternative protein sources and their inclusion levels need to be optimized in fish diets to make aquaculture production more efficient and cost-effective. Digestibility trials are essential to shed light on the potential of these ingredients for fish before their inclusion in diet formulations.

There are numerous factors involved in the digestibility performance of common feed ingredients for fish. Digestibility coefficients are also different for the same ingredient depending on the species under study (Davies et al., 2009). Apparent digestibility coefficient (ADC) values of feed ingredients are normally the result of differences in species, changes in the harvest/catch season of the raw materials, and conditions under which they are processed. For that reason, studies on various marine fish species have been conducted (Booth et al., 2005; Davies et al., 2009; Lupatsch et al., 1997; Tibbetts et al., 2004, 2006) to assess the quality value of different ingredients.

Taking into account the relevance of digestibility estimation for diet formulation, the aim of this study was to compare the apparent

nutrient digestibility and protein quality of 12 different commercially available ingredients for *S. dumerili*.

2. MATERIAL AND METHODS

2.1. Ingredients and experimental diets

Yellowtail (*S. dumerili*) juveniles were caught in the Mediterranean and transferred to the facilities of the Polytechnic University of Valencia, Spain.

Feed ingredients were tested for apparent protein digestibility, availability of digestible amino acids, and protein quality in fish meal, krill meal, squid meal, poultry meal (international feed number 5-03798), defatted krill meal (a product obtained by removing the fat with ethanol), soybean (*Glycine max*), wheat gluten (*Triticum spp*), camilina (*Camelina sativa*) (product given to our facilities by local farmers), pea (*Pisum sativum*), sunflower (*Helianthus annuus*), and fava bean (*Vicia faba*) meals (Tables 4.1 and 4.3). Test diets were prepared by mixing a basal diet with one of the test ingredients (Table 4.2) in a ratio of 7:3, as described by Cho & Slinger (1979).

The different ingredients of the diets were weighed individually and mixed to form homogeneous dough and were prepared through cooking-extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). The conditions under which they were processed are as follows: a screw speed of 100 rpm, a temperature of 110 ºC, and a pressure of 40–50 atm. The experimental diets were assayed in quintuplicate. The fish were handfed once a day (8:00 h), and fecal collection occurred 8 hr later (16:00 h). The pellet size used was 3 mm during the experiment, increasing to 4 mm in the final part of the experiment. The pellets were slowly distributed to give all fish time to eat. The uneaten diet was collected when fish had finished feeding.

2.2. Digestibility assay

Fifteen yellowtails (175 ± 2.8 g initial mean live weight) were randomly distributed in five experimental tanks (190 L fiberglass tanks, 88 cm length, 62 cm diameter, and 188 cm depth) of a marine semiclosed recirculating system designed based on the Guelph system (the fecal material being collected in a settling column). Each tank was loaded with three fish. The velocity of the water flow was adjusted to minimize settling of the feces in the drainpipe and maximize feces recovery in the settling column.

There was a 3-month adaptation period (adaptation to dry diets was a very long process, in which yellowtail were first fed anchovy followed by a semimoist diet—a mix of anchovy and fish meal—before being fed the control diet). Once the fish had become accustomed to both the tanks and the dietary regime, collection of feces was initiated.

Table 4.1 Proximate composition of ingredients used in the digestibility trial (expressed as g/kg of dry matter basis, dm).

Ingredients[a] (g/kg)	Dry matter	Crude Protein	Crude Lipid	Ash	N-free Extract
Plant protein ingredients					
Fava bean meal	890	237	12	33	718
Camilina meal	918	391	45	61	503
Extracted soybean meal	882	499	22	71	408
Pea meal	866	216	10	39	736
Sunflower meal	896	291	15	67	627
Wheat meal	890	116	15	18	851
Wheat gluten	933	810	9	9	173
Animal protein ingredients					
Extracted krill meal	878	723	24	102	151
Fish meal	903	713	93	168	26
Krill meal	888	561	225	104	111
Meat meal	970	531	153	269	47
Squid meal	928	719	30	110	142

[a] Wheat (*Triticum* spp), Sunflower (*Helianthus annuus* L.), pea (*Pisum sativum* L.), fava bean (*Vicia faba* L.) and soybean meal (*Glycine max* L. Merr.) were provided by DESCO, Museros, Valencia (Spain); Extracted krill meal was provided by LUDAN RENEWABLE ENERGY, Valencia (Spain); Wheat gluten and Camilina (Camelina sativa, L. Crantz) were provided by DADELOS AGRÍCOLA, Valencia (Spain); Meat meal was provided by VALGRA S.A., Beniparrell, Valencia (Spain); Krill meal was provided by AKER SEAFOODS ANTATARCTIC, S. A. Lysaker (Norway); Squid meal was provided by MAX NOLLERT, Utrecht (Netherlands).

Table 4.2 Formulation and apparent digestibility coefficients of the experimental diets.

		Plant protein ingredients							Animal protein ingredients			
Test ingredients (g/kg)	**Control**	**Fava bean meal**	**Camilina meal**	**Soybean meal**	**Pea meal**	**Sunflower meal**	**Wheat gluten**	**Deffated krill meal**	**Fish meal**	**Krill meal**	**Poultry meal**	**Squid meal**
Fava bean meal		300										
Camilina meal			300									
Soybean meal				300								
Pea meal					300							
Sunflower meal						300						
Wheat meal	240	168	168	168	168	168	168	168	168	168	168	168
Wheat Gluten							300					
Deffated krill meal								300				
Fish meal	665	465.5	465.5	465.5	465.5	465.5	465.5	465.5	765.5	465.5	465.5	465.5
Krill meal										300		
Poultry meal											300	
Squid meal												300
Fish oil	75	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5
Multivitamin and mineral mix	20	14	14	14	14	14	14	14	14	14	14	14
Crude protein (% ww)	46.2	37.4	41.3	40.3	35.8	37.4	55.2	56.0	53.0	45.4	51.3	49.5
Crude lipids (% ww)	12.2	9.0	10.1	8.8	8.7	9.0	9.0	13.0	13.9	13.8	14.8	10.9
AIA (g/kg dry matter)	2.2	2.2	3.1	3.1	2.8	1.6	1.8	2.0	3.5	3.4	3.6	3.0
Dry matter ADC (%)	84.8	72.1	76.4	75.8	76.5	78.9	84.1	78.6	83.9	76.6	80.2	81.0
Protein ADC (%)	96.9	85.8	83.6	90.4	88.5	90.2	94.5	93.5	97.5	90.0	93.8	96.1
Lipid ADC (%)	98.5	96.2	97.5	94.9	96.1	97.2	98.2	96.6	98.2	96.8	98.1	96.7

The fish were fed one meal a day at 10.00 h. The feed was offered only as long as the fish were actively feeding in order to avoid wastage. One hour after the meal, the drainpipe and the settling column were brushed out to remove uneaten feed particles and feces from the system to avoid mixing feed particles and feces in the recollection. Feces were collected the next day fromthe base of the settling column into a plastic container by gravity at 8:00 h. After feces collection, the fish were fed again at 10:00 h, waiting 2 h between feces recollection and feeding to avoid stress.

The water temperature was maintained around 20 ºC (21.5 ± 2.4 ºC) during the experimental period by a water conditioning pump (TRANE CAN 490, 123.3 kW) installed in the system. All tanks were equipped with aeration, and the level of dissolved oxygen was 6.6 ± 1.3 mg/L. Water salinity was 31.5 ± 4.1 g/L, pH 7.3 ± 0.4, NO_{3-} (25–150 mg/L), and NO_{2-} (0.05–0.5 mg/L), and the ammonium value was undetectable. The photoperiod was maintained at 12-h light and 12-h dark by means of artificial daylight simulation. All these parameters were measured daily from Monday to Saturday. These parameters are optimal for *S. dumerili* in the recirculation system and are similar to other Seriola experiments (Jover et al., 1999; Monge-Ortiz et al., 2018a, 2018b).

Over a 6-month period, the fish groups were fed the experimental diets, and feces were sampled from each tank. One tank was fed with the control diet for over 6 months to determine the possible variations in digestibility throughout the experimental period. Experimental periods were 14 days long for each diet and each replicate. On the last 3 days of the 2-week period, fish were fasted in order to avoid mixing feces between diets. Feces were removed immediately to determine their amount and have enough for analysis, following which fish continued to be fed the same diet for another week. The switching of dietary treatments was as follows in the experimental trial: diets with fava bean, camilina, soybean, and pea meal were tested from week 1 to week 2, from 7 to 8, from 13 to 14, and from 19 to 20, respectively; diets with sunflower, wheat, wheat gluten, and defatted krill meal were assayed from week 3 to week 4, from 9 to 10, from 15 to 16, from 21 to 22, and from 25 to 26, respectively; and diets containing fish,

krill, meat, and squid meal were tested from week 5 to 6, from 11 to 12, from 17 to 18, from 23 to 24, and from 27 to 28, respectively.

The fecal material collected was dried to a constant weight in an oven at 60ºC for 48 hr prior to analysis and stored in airtight plastic containers until nutrient component and inert marker (acid insoluble ash, AIA) analysis. The AIA content of feeds and feces was determined using the method of Atkinson et al. (1984) to calculate the ADC.

The ADCs (ADC_{diet},%) of each specific nutritional variable (protein (ADC_p, %), lipid (ADC_l, %), and AA) of the diets were calculated using the following formulae:

$$\mathrm{ADC_{diet}} = 1 - \frac{\mathrm{marker_{diet}\ x\ nutrient_{faeces}}}{\mathrm{marker_{faeces}\ x\ nutrient_{diets}}}$$

In this equation, the terms $marker_{diet}$ (g/kg) and $marker_{faeces}$ (g/kg) represent the marker content of the diet and the feces, respectively, and $nutrient_{diet}$ (g/kg) and $nutrient_{faeces}$ (g/kg) are the nutritional parameters of concern (e.g., protein or energy) in the diet and the feces, respectively.

The ADCs of dry matter, protein, lipid, and amino acids for the test ingredients (30% replacement level) were calculated as follows:

$$\mathrm{ADC_{ing}} = \frac{[(\mathrm{a} + \mathrm{b})\ \mathrm{x}\ \mathrm{ADCN_{test}} - \mathrm{a}\ \mathrm{x}\ \mathrm{ADCN_{ref}}]}{\mathrm{b}}$$

In the equation above, "a" is the nutrient contribution of the reference diet to nutrient content of the test diet, "b" is the nutrient contribution of test ingredients to the nutrient content of the combined diet, and "a + b" is the level of nutrient in the combined diet (%); $ADCN_{test}$ (%) = ADC of a nutrient in the combined diet and $ADCN_{ref}$ (%) = ADC of a nutrient in the reference diet (Forster, 1999).

2.3 Chemical analysis

A chemical analysis of the dietary ingredients was performed prior to diet formulation (Table 4.1). Diets and their ingredients were analyzed according to (AOAC, 1995) procedures: dry matter (105 ºC to constant

weight), ash (incinerated at 550 ºC to constant weight), and crude protein (N x 6.25), assessed by the Kjeldahl method after acid digestion (Kjeltec 2300 Auto Analyzer, Tecator Höganas, Sweden), and crude lipid, extracted with methyl-ether (Soxtec 1043 Extraction Unit, Tecator, Höganas, Sweden).

Following the aforementioned method by Bosch et al. (2006), the amino acid content of the fish carcasses and the diets were established using a Waters HPLC system (Waters 474, Waters, Milford, MA) with two pumps (Model 515, Waters), an autosampler (Model 717, Waters), a fluorescence detector (Model 474, Waters), and a temperature control module. Before hydrolyzation, alpha aminobutyric acid was added as an internal standard. The derivatization of amino acids was made using AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). Methionine and cysteine were determined separately as methionine sulphone and cysteic acid after oxidation with performic acid. Amino acids were separated with a C-17 reverse-phase column Waters Acc. Tag (150 mm × 3.9 mm) and subsequently transformed to Met and Cys.

2.4 Protein quality

The values of the following indices were calculated:

• Amino acid ratio (AAR, %) = $(AA_{sample})/(AA_{reference}) \times 100$, where AA_{sample} and $AA_{reference}$ are the digestible amino acid contents in the test sample and whole *S. dumerili* (Figure 4.1), respectively, the one taken as reference (average values of 20 samples of fish weighing 100–500 g).

• Chemical score (CS, %): minimum value from AARs calculated for digestible essentials amino acids (EAA: Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val).

• Limiting amino acid: the digestible amino acid corresponding to CS in the test sample.

• Oser's Index (OI, %): used as index of nutritional quality and achieved as the geometric mean ratio of digestible amino acids in samples of the ones detected in *S. dumerili*, which were taken as a reference according to the following formula:

$$\mathrm{OI}\ (\%) = \left(10^{\left(\frac{1}{n}\,x\,(\log(\mathrm{ARR}_1)+\log(\mathrm{ARR}_2)+\cdots\log(\mathrm{ARR}_n)\right)}\right)$$

in which AAR1, AAR2, …AARn are the ratios of digestible essential amino acids, and "n" is the number of detected digestible essential amino acids. When the ratio was above 100, this was taken as reference (Oser, 1951).

2.5 Statistical analysis

Digestibility coefficients were evaluated using one-way analysis of variance (ANOVA). ACD data were $\log_{10}$ transformed to meet the assumptions of statistical tests (normality, linearity, and homoscedasticity). The Newman–Keuls test was used to assess specific differences among diets at a level of $p < 0.05$ (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, VA).

3 RESULTS

3.1 Proximate composition and amino acid content

The proximate composition of the 12 test feed ingredients is reported in Table 4.1. The greatest protein values were observed in animal meals: fish meal (713.2 g/kg), wheat gluten (810 g/kg), squid meal (718.8 g/kg) and extracted krill meal (723.1 g/kg), while the lowest were found in vegetable meals, such as pea (215.9 g/kg), fava bean (236.6 g/kg), and sunflower meal (291.3 g/kg).

Vegetal meals presented the poorest amino acid balance, with serious deficiencies in EAA, such as in arginine, threonine, lysine, and methionine (Table 4.3). Arginine, lysine, and leucine were the predominant essential amino acids in animal ingredients, of which fish

and defatted krill meal had a relatively similar content. The principal nonessential amino acids present in animal and vegetal meals were aspartate and glutamine.

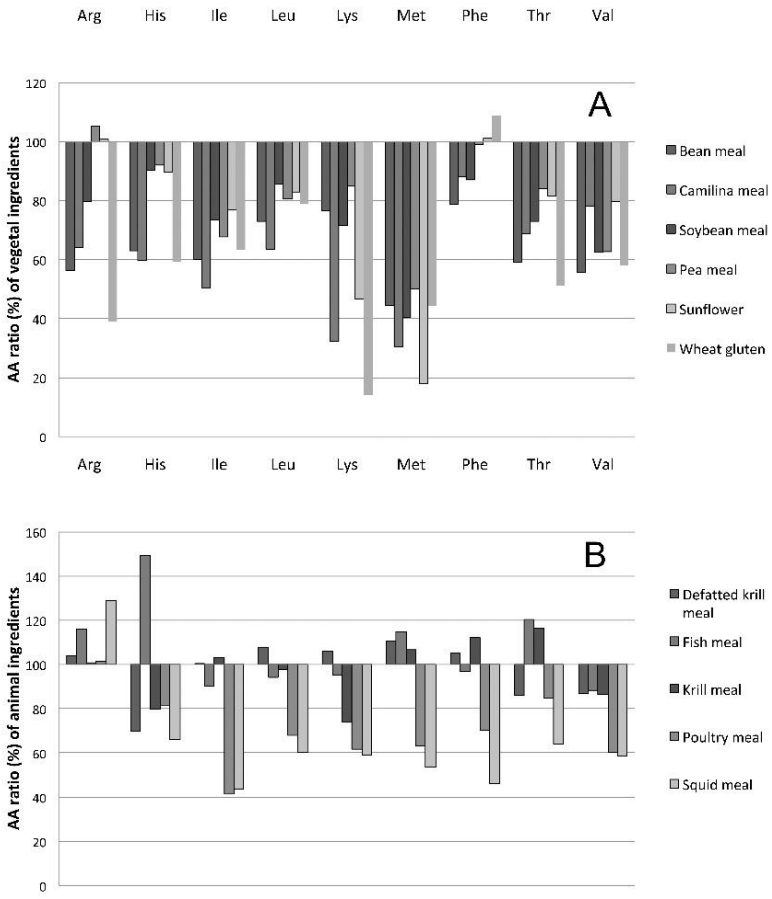

Figure 4.1 Amino acid ratios (%) for esential digestible amino acids in vegetable ingredients (A) and in animal ingredients (B). Essential amino acids (EAA) of the whole body of *Seriola dumerili* were used to estimate this index as AA reference (expresed in g/100g of protein): Arg (7.14), His (2.65), Iso (4.76), Leu (7.50), Lys (8.14), Met (2.37), Phe (4.32), Thr (3.85), Val (5.60).

3.2 Digestibility

The ADCs of dry matter for yellowtail ranged from 71.88 to 89.53% for animal ingredients and from 65.89 to 79.98% for plant products. The

higher-protein ADCs were observed in fish meal (91.3%), wheat gluten (87%), defatted krill meal (86.7%), squid meal (85.2%), and poultry meal (85.0%); mid-range in krill meal (75.8%), soybean meal (73.2%), and sunflower and pea meal (63%); and low in camilina (48.5%) and fava bean meal (54.9%) (Table 4.4). ADCs of lipids in all treatments were above 88%, except in the case of pea meal (83.5%)

For the animal ingredients, the availability of amino acids (Table 4.5) in fish, squid, meat, and defatted krill meal was generally higher than those in krill meal. Among all the plant ingredients, the availability of amino acids in wheat gluten was the highest, followed by soybean, pea, and sunflower meal. Camilina and bean meal presented the lowest AA ADCs.

3.3 Protein quality evaluation

Defatted krill meal was the most balanced digestible protein ingredient (Figures 4.1 and 4.2). It was only deficient in histidine, threonine, and valine (AAR: 70 [CS], 86, and 87%, respectively). Fish meal was slightly deficient in isoleucine, leucine, lysine, phenylalanine, and valine (AAR: 90, 94, 95, 97, and 88%, respectively). Krill meal was deficient in histidine, lysine, and valine (AAR inferior to 90%). Squid and poultry meals presented 60–85% ARRs in all the digestible amino acids, except for arginine, which was the most balanced of them all, while isoleucine was the least balanced (AAR < 50%). With respect to the vegetal ingredients, wheat gluten showed the lowest CS (13.98% for lysine), followed by sunflower, which was found to be deficient in Met (CS: 17.97%), and camilina meal (poor in methionine, CS: 30.8%). Pea meal proved to be a balanced amino acid ingredient (its CS, met, was above 50%). OI (Figure 4.2), calculated for protein meals, was positively related with CS, presenting the lowest values of OI for those that presented the lowest CS, such as wheat meal and sunflower meal (50.41% OI, 13.89% CS and 67.96% OI, 17.97% CS respectively). On the contrary, defatted krill and fish meal (92–96%) showed the most balanced protein with regard to essential amino acids, whereas all the other ingredients presented lower levels (50–78%).

Table 4.3 Amino acid composition of ingredients (expressed as g/kg of dry matter basis, dm).

	Plant protein ingredients							**Animal protein ingredients**				
	Favabean meal	**Camilina meal**	**Soybean meal**	**Pea meal**	**Sunflower meal**	**Wheat meal**	**Wheat gluten**	**Deffated krill meal**	**Fish meal**	**Krill meal**	**Poultry meal**	**Squid meal**
EAA (g/kg)												
Arginine	17.7	36.7	32.3	15.2	27.7	4.6	257	53.9	60.5	42.2	37.2	63.3
Histidine	6.6	10.3	12.5	5.0	8.4	2.4	14.5	13.0	29.1	12.8	10.8	12.0
Isoleucine	9.2	15.1	20.5	8.5	14.7	3.6	30.1	34.2	32.2	32.5	10.1	15.2
Leucine	18.1	28.6	37.2	15.4	24.7	6.7	57.9	57.5	55.5	47.4	26.2	32.6
Lysine	17.0	17.9	30.5	16.6	13.7	3.2	12.1	58.0	56.9	38.4	25.6	33.8
Methionine	2.8	3.2	8.1	3.1	1.8	6.3	8.8	17.5	20.2	16.8	7.5	8.4
Phenylalanine	9.8	19.1	23.0	9.6	16.2	4.5	43.1	33.2	30.8	30.3	15.8	15.5
Threonine	8.3	16.9	17.5	7.5	13.0	3.2	19.5	31.1	34.2	27.8	16.8	18.0
Valine	10.1	22.4	20.3	9.2	18.0	4.7	32.6	35.6	37.6	31.7	17.6	23.6
NEAA (g/kg)												
Alanine	9.8	18.8	19.0	8.3	14.8	3.5	20.0	41.4	45.8	33.1	36.8	52.6
Aspartate	25.9	39.4	57.7	23.6	43.6	5.4	22.3	81.0	65.1	60.3	50.8	46.0
Cystine	2.1	4.5	4.1	2.0	2.9	7.1	11.2	3.5	6.6	3.8	7.3	35.8
Glutamine	41.3	76.8	94.1	36.7	74.9	28.9	319.8	110.5	92.2	75.2	69.9	102.6
Glycine	10.2	23.3	18.6	8.4	20.2	4.7	24.5	30.6	48.3	27.8	75.4	106.9
Proline	8.9	22.7	21.7	7.4	16.0	9.4	108.2	23.8	29.7	23.0	42.7	53.6
Serine	12.1	17.3	24.2	9.6	15.4	4.9	36.7	29.1	29.9	24.8	22.8	22.9
Tyrosine	4.2	9.7	12.4	3.5	6.7	1.3	22.9	29.3	23.0	27.3	11.2	11.8
EAA/NEAA	0.87	0.80	0.80	0.91	0.71	0.60	0.43	0.96	1.05	1.02	0.53	0.51

EAA: Essential amino acids; NEAA: Nonessential amino acids.

Table 4.4 Apparent digestibility coefficients (ADCs) for dry matter, crude protein, and crude lipid in test ingredient consumed by yellowtail.

	Plant protein ingredients						**Animal protein ingredients**					
ADC (%)	Fava bean meal	Camilina meal	Soybean meal	Pea meal	Sunflower meal	Wheat gluten	Deffatted krill meal	Fish meal	Krill meal	Poultry meal	Squid meal	SEM
Dry matter	42.2^{d}	58.8^{c}	54.9^{c}	57.2^{c}	65.7^{c}	82.2^{ab}	68.0^{c}	87.2^{a}	57.7^{c}	70.2^{bc}	70.1^{bc}	3.4
Protein	54.9^{d}	48.5^{e}	73.2^{c}	63.0^{d}	62.9^{d}	87^{b}	86.7^{b}	91.3^{a}	75.8^{c}	85.0^{b}	85.2^{b}	1.3
Lipid	90.2^{ab}	91.3^{ab}	91.1^{ab}	83.5^{c}	94.0^{a}	90.8^{ab}	88.4^{b}	94.2^{a}	92.0^{ab}	93.9^{a}	88.3^{b}	1.2

Note. Data log with different letters in the same line denote significant differences ($p < 0.05$). Newman-Keuls test.

Table 4.5 Apparent digestibility coefficients (ADCs) of aminoacids in test ingredients consumed by yellowtail.

	Plant protein ingredients						Animal protein ingredients				
ADC (%)	**Fava bean meal**	**Camilina meal**	**Soybean meal**	**Pea meal**	**Sunflower meal**	**Wheat gluten**	**Deffated krill meal**	**Fish meal**	**Krill meal**	**Poultry meal**	**Squid meal**	**SEM**
EAA												
Arginine	41.0^{g}	37.7^{h}	68.8^{f}	79.2^{d}	72.3^{e}	84.1^{c}	89.1^{a}	90.0^{a}	86.6^{b}	82.7^{c}	90.3^{a}	0.8
Histidine	47.4^{e}	42.9^{ef}	85.4^{b}	77.0^{cd}	81.0^{bc}	77.6^{cd}	90.2^{a}	90.3^{a}	81.8^{bc}	91.5^{a}	92.1^{a}	1.5
Isoleucine	53.6^{e}	39.7^{f}	68.8^{d}	64.4^{d}	69.3^{d}	78.0^{c}	89.0^{a}	91.0^{a}	80.7^{bc}	85.8^{ab}	85.5^{ab}	1.5
Leucine	51.5^{f}	46.7^{g}	72.6^{d}	63.4^{e}	70.6^{d}	79.4^{c}	85.4^{b}	87.6^{ab}	77.0^{c}	91.4^{a}	90.9^{a}	1.2
Lysine	64.2^{d}	41.2^{e}	79.0^{bc}	69.9^{cd}	78.7^{bc}	70.1^{cd}	91.6^{ab}	93.7^{a}	78.1^{bc}	83.8^{ab}	90.7^{ab}	2.5
Methionine	61.4^{f}	65.5^{e}	47.4^{g}	66.5^{e}	63.3^{ef}	93.5^{ab}	86.6^{c}	92.0^{b}	72.3^{d}	95.4^{a}	88.2^{c}	0.9
Phenylalanine	57.1^{f}	57.1^{f}	65.1^{e}	78.1^{d}	81.0^{d}	85.2^{c}	87.0^{bc}	93.6^{a}	80.7^{d}	89.3^{b}	80.4^{d}	1.0
Threonine	45.8^{d}	50.2^{d}	62.6^{c}	64.1^{c}	67.8^{c}	79.7^{b}	69.2^{c}	90.0^{a}	84.1^{ab}	90.8^{a}	93.1^{a}	2.3
Valine	53.1^{c}	55.2^{c}	69.7^{bc}	63.2^{bc}	70.7^{bc}	76.3^{ab}	86.5^{a}	92.0^{a}	88.0^{a}	90.4^{a}	90.8^{a}	4.3
NEAA												
Alanine	61.6^{e}	49.2^{f}	69.4^{d}	59.9^{e}	73.3^{cd}	79.4^{bc}	85.2^{ab}	91.7^{a}	79.8^{bc}	91.2^{a}	91.3^{a}	2.2
Aspartate	40.4^{d}	63.5^{c}	73.6^{bc}	72.7^{c}	70^{c}	80.0^{ab}	84.7^{a}	91.7^{a}	82.7^{a}	88.8^{a}	86.7^{a}	2.6
Cystine	57.7^{c}	60.6^{c}	35.6^{d}	75.2^{abc}	62.9^{bc}	95.3^{a}	82.5^{abc}	91.0^{ab}	67.6^{bc}	75.7^{abc}	94.8^{a}	6.4
Glutamine	62.6^{c}	45.9^{d}	84.6^{a}	64.3^{c}	74.2^{b}	85.1^{a}	88.0^{a}	93.3^{a}	65.9^{c}	84.9^{a}	87.9^{a}	2.7
Glycine	48.6^{c}	42.2^{d}	68.7^{b}	69.0^{b}	69.3^{b}	88.9^{a}	88.4^{a}	92.3^{a}	71.8^{b}	88.8^{a}	95.1^{a}	2.0
Proline	55.5^{d}	46.6^{e}	66.2^{c}	73.8^{bc}	68.3^{c}	88.4^{a}	82.6^{ab}	90.3^{a}	83.4^{ab}	85.3^{ab}	87.9^{a}	3.0
Serine	55.1^{e}	49.9^{f}	69.2^{c}	71.1^{c}	64.5^{d}	81.0^{b}	90.1^{a}	86.3^{ab}	78.9^{b}	83.6^{ab}	91.0^{a}	2.2
Tyrosine	60.8^{f}	53.9^{g}	66.4^{e}	70.5^{d}	75.3^{c}	85.1^{b}	88.4^{a}	89.3^{a}	84.7^{bc}	84.4^{a}	84.7^{a}	0.8

4. DISCUSSION

The results obtained in the present study confirmed the very high digestibility of certain animal proteins in carnivorous marine fish (Lupatsch et al., 1997; Tibbetts et al., 2006). ADCs of animal proteins were close to those obtained for fish meal regarding crude protein digestibility, and that fact is probably the result of their very high protein concentrations. Only krill meal's ADCp was lower than the other animal protein sources, probably because of the unusually high amount of fat of the krill meal sample used in this study (and consequent low protein level). This was the main difference between the krill meal and the defatted krill meal, which has a lower fat and a higher protein level, making it a higher-quality product for yellowtail diets. Tibbetts et al. (2006) obtained similar results with shrimp, with low ADC, because of the high ash content of the shrimp meal.

Protein ADCs of fish meal assessed in yellowtail are also similar to the ones encountered in turbot (*Psetta maxima*), sea bass (*Dicentrarchus labrax*), sea bream (*S. aurata*), Atlantic cod (*Gadus morhua*), red drum (*Sciaenops ocellatus*), Australian snapper (*Pagrus auratus*), and Atlantic halibut (*Hippoglossus hippoglossus*), at 91–96% (Booth et al., 2010; Burel et al., 2000; Davies et al., 2009; Gaylord & Gatlin, 1996; Gomes da Silva & Oliva-Teles, 1998; Lupatsch et al., 1997; Peach, 2005; Tibbetts et al., 2006).

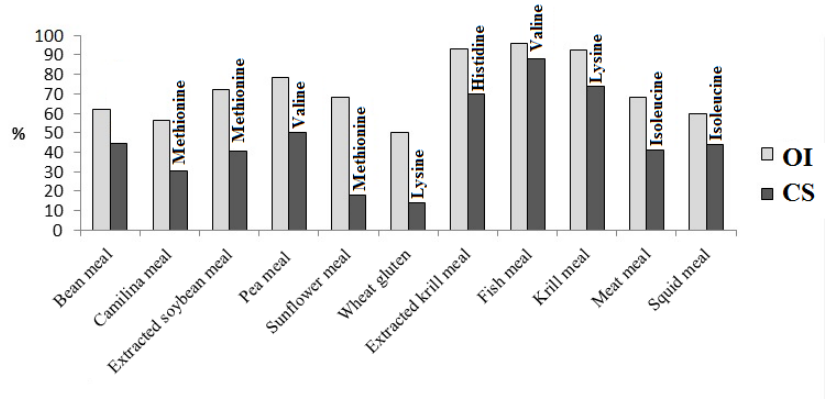

Figure 4.2 Oser´s index (OI), Chemical Score (CS) and limiting amino acid in test ingredients for digestible amino acids.

Protein ADCs were high for poultry meal (85%). Animal byproduct meals can vary greatly in their proximate composition, depending on several factors (raw material source and freshness, production processes, and storage), and because of its very nature, the values reported for protein ADCs are also highly variable in studies on fish. In many fish species, poultry meal has received positive results in diets (ADCp 85.5% in sea bass, 79–80% in sea bream, 78.4% in turbot, and 80% in Atlantic cod) and is regarded as the most effective animal protein (Davies et al., 2009; Lupatsch et al., 1997; Tibbetts et al., 2006).

The ADCp of krill hydrolysate found in Atlantic halibut (Peach, 2005) and Atlantic cod (Tibbetts et al., 2006) was nearly 100%, very different indeed to the krill ADCp in yellowtail (76%), but that krill meal was obtained through finely grinding whole krill that had previously been freeze-dried (*Euphausia superba*), and therefore, the proximate composition was fairly different from the one found in commercially produced krill meals like the one used in the present work. Those discrepancies are explained by the differences in product quality, as in the case of the defatted krill meal (full-fat krill meal) with a higher protein level.

There are numerous studies on digestibility of soybean meal on various fish species. The protein ADC, however, varied a great deal (76–98%). Yellowtail is within this range, although on the lower side, that is, it is among the species that present a lower soybean protein digestibility. Other authors observed a decrease in protein digestibility (Refstie et al., 1999; Robaina et al., 1995), mainly attributed to the presence of phytate.

Protein ADC was high for wheat gluten meal (87%) but inferior to that reported for other marine fish (100%), like sea bass (Robaina et al., 1999) and Atlantic cod (Tibbetts et al., 2006). The high palatability and digestibility, together with the absence of antinutritional factors, make wheat gluten meal, supplemented with some amino acids (lysine, methionine and arginine), a suitable fish meal replacement candidate in yellowtail diets.

Pea meal had a midrange ADCp. This ingredient appears to have some potential for use in marine fish diets (Sánchez-Lozano et al., 2009,

2011); nonetheless, it should be pre-extruded in order to boost the digestibility of nonprotein ingredients.

Lipid ADC results indicate that lipids from both animal and plant sources were well digested by *S. dumerili*. There were only slight differences in this parameter between soybean meal and most of the raw materials. Several studies have concluded that dietary soybean meal decreases the lipid digestibility in salmonids (Romarheim et al., 2008). This fact must be attributed to the bile acid level, which is reduced by dietary soybean meal (Romarheim et al., 2006; Yamamoto et al., 2007).

Regarding the availability of AAs, it should be noted that the digestibility of each AA within a feed ingredient is variable. This variability, nonetheless, increases in those ingredients with a lower protein ADC (AA ADCs of camilina or fava bean meals were between 38 and 66%). In general, ADCs of AAs reflect the apparent CP digestibility coefficients of some test ingredients. In some of them, however, there are differences, for example, in wheat gluten, where the ADCs of AAs were lower than ADCp. On the contrary, krill meal, pea meal, or poultry meal presented higher AA ADCs. Thus, amino acid availabilities cannot always be estimated from the ingredient ADCp. Fish meal and defatted krill meal are the most balanced digestible amino acid ingredients and have one advantage: they benefit from having a higher protein content and CS than krill. However, the defatted krill meal also has one shortcoming: the need for histidine supplementation. Regarding other animal sources, squid meal was the most balanced amino acid in arginine, and poultry meal protein quality was similar to camilina and soybean meal.

Despite the high protein digestibility observed in the present work for some protein meals, the biological value of the protein should also be taken into account before diet formulation to include them in fish diets. For example, wheat gluten digestibility was very high; however, it shows a low OI and the lowest CS (in this case lysine), and therefore, it cannot replace the fish meal in high dietary levels for Mediterranean yellowtail feeding.

It has been observed in studies performed with sea bream (Lupatsch et al., 1997) that, when using ACDp values obtained for individual ingredients to calculate the ACDp for compound diets, both values were very similar, and consequently, protein digestibility can be considered to be additive. Therefore, ACDp results obtained in this present experiment, along with the protein quality assessments, should be taken into account when formulating diets for Mediterranean yellowtail. This is a useful tool to simulate the ideal protein in diets because, by combining different ingredients, the inclusion of synthetic amino acids could be avoided or diminished, which would reduce the cost of fish diets and improve protein digestion.

In conclusion, animal sources are evidently suitable as protein ingredients in yellowtail diets, although the protein quality indices present in them could be enhanced through some essential amino acid supplementation. None of the vegetal meals assayed could offer a good nutritional balance on their own, and therefore, they would either need to be supplemented or need to be used together with other raw materials.

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Capítulo 5

Partial and total replacement of fish meal by a blend of animal and plant proteins in diets for *Seriola dumerili*: Effects on performance and nutrient efficiency.

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ABSTRACT

A 154-day trial was performed to assess the use of an alternative protein blend (corn gluten, krill and meat meal) as a substitute for fish meal in diets for juvenile yellowtail, using four isolipidic (140 g/kg) and isoenergetic diets (24 MJ/kg) with the same digestible protein content (50%). The control diet was FM100, without replacement, and in FM66, FM33 and FM0, fish meal was replaced at 33 g/kg, 66 g/kg and 100 g/kg respectively. At the end of the experiment, no differences in growth parameters were observed. Fish fed the FM0 diet exhibited the lowest survival (23%). This high mortality may be due to different factors, such as a dietary amino acid imbalance or some antinutrient factors contained in the alternative ingredients. Feed intake, feed conversión ratio, digestible protein intake and protein efficiency ratio were similar in all diets. However, digestible energy intake and protein efficiency retention were lowest in fish fed the FM0 diet. Apparent digestibility coefficients for protein, energy and amino acids diminished as a substitution for fish meal increased. Significant differences were observed in the diet whole-fish body profile amino acid retention ratio for the seven essential amino acids. In summary, total fish meal replacement by the blend assayed was not feasible for yellowtail. The FM66 diet resulted in good growth, high survival and good nutrient efficiency.

1. INTRODUCTION

Its fast growth, excellent meat quality and its recent reproduction in captivity make the Mediterranean yellowtail (*Seriola dumerili*, Risso 1810) one of the most promising species for Mediterranean aquaculture to enhance the diversity of fish production. However, to maintain a profitable commercial culture of the Mediterranean yellowtail, it is necessary to formulate diets with alternative ingredients for economic and environmental benefits to avoid the high use of fish meal in the diets.

Tacon & Metian, (2008) reported that 75% of the world fish stocks are currently considered fully exploited or overexploited, including many small pelagic fish species used to produce fish meal for feed formulation worldwide. As fish meal production is predicted to be unable to sustain the growth of the aquaculture sector, the quest for alternative ingredients and protein sources and the optimization of dietary protein content are important goals. Studies carried out on the effects of alternative protein inclusion in diets for the Mediterranean yellowtail are scarce. At present, there are few studies on the subject. Tomás et al., (2005) reported a range of 20 g/kg to 30 g/kg as the maximum level of soybean dietary inclusion for the Mediterranean yellowtail as the higher inclusion levels led to poor growth and poor nutrient efficiency. Uyan et al., (2009) reported that the dietary phospholipid supplementation in non-fish meal diets could increase feed intake and improve growth of *S. dumerili*.

Protein quality is generally evaluated according to its amino acid profile and the bioavailability of its amino acids (AAs). The ideal dietary AA profile could be obtained using an appropriate mixture of proteins and, thus, a high fish meal substitution could be achieved successfully, as reported for other carnivorous species (Espe et al., 2006; Kissil & Lupatsch, 2004; Monge-Ortiz et al., 2016; Sánchez-Lozano et al., 2009, 2011). Nevertheless, high or total fish meal dietary replacement may produce undesirable effects on fish, caused mainly by the antinutrients particularly contained in vegetable proteins (Francis et al., 2001). These antinutrients may decrease enzyme activity, lower protein digestibility (Spinelli et al., 1983) and enhance susceptibility to

pathogenic infections (Maita et al., 1998) and mortality (Estruch et al., 2015) due to immunosuppression (Sitjà-Bobadilla et al., 2005). One of the solutions for reducing these antinutrients in diets could be to substitute vegetal protein for animal proteins, which in turn can alleviate the deficiencies of amino acids, decrease supplementation with synthetic AAs (Lu et al., 2015) and reduce the lack of palatability and "green liver" (Watanabe et al., 1995). As particular metabolic functions require specific amino acids (AAs) and body-building depends upon the ingestion of components of structural proteins, it is crucial that fish ingest, digest and bio-assimilate the necessary AAs from protein sources. Diet amino acids must be balanced, using the first approximation to the ideal amino acid profile, the fish muscle, as essential AA (EAA) requirements and muscle AA of fish present a high degree of correlation (Peres & Oliva-Teles, 2008).

The animal proteins (APs) for use in aquafeeds in the European Union (EU) have been permitted since 2013 when the APs are not derived from ruminants. In this case, we have selected defatted krill meal and meat meal as the main substitution protein for *S. dumerili* feeds. The defatted krill used in this study is a by-product obtained from the krill oil extraction for human consumption, so it has a very suitable price (0.25 €/kg) to be used in diets for fish instead of fish meal (1.51 €/kg). The non-ruminant meat and bone meal (meat meal) is an animal by-product that derives from slaughterhouse leftovers, being manufactured worldwide with a steady availability, averaging a production of 3.5 million tons per year in the EU (Coutand et al., 2008; Moutinho et al., 2016).

To date, experimental cultures aimed at the development of commercial-scale production of the Mediterranean yellowtail in the Mediterranean have often failed due to the parasite and/or pathogenic infections, particularly in juvenile fish. Considering the negative effects of fish meal replacement, the design of specific diets suitable for the optimal rearing of the Mediterranean yellowtail is crucial.

Considering these facts and the scarcity of nutritional studies on the effects of fish meal replacement in diets for the Mediterranean

yellowtail, the aim of this work was to assess fish meal replacement by a blend of feedstuffs in diets for this species, not only regarding growth, but also evaluating nutrient and amino acid efficiency.

2. MATERIALS AND METHODS

2.1. Production system

The trial was carried out in 12 cylindrical fibreglass tanks (1750 L) inside a recirculated seawater system (75 m^3 capacity) with a rotary mechanical filter and a gravity bio-filter (approximately 6 m^3 capacity) at the Aquaculture Laboratory (Animal Science Department at Polytechnic University of Valencia, Valencia, Spain). The marine water in the system was changed once every 3 months.

The experimental period was 154 days (from January to June of 2014). The water temperature was maintained at 21.5 ± 2.4°C during the experimental period by a water conditioning pump (TRANE CAN 490, 123.3 kW) installed in the system. All tanks were equipped with aeration, and the level of dissolved oxygen was 6.6 ± 1.3 mg/L. Water salinity was 31.5 ± 4.1 g/L, pH 7.3 ± 0.4, NO_{3-} (25–150 mg/L), and NO_{2-} (0.05– 0.5 mg/L), and the ammonium value was undetectable. The photoperiod was natural throughout the experimental period (16L/8D in summer and 12L/12D in winter), and all tanks had similar lighting conditions. All these parameters were measured daily from Monday to Saturday.

2.2 Fish and experimental design

Mediterranean yellowtail (*Seriola dumerili*) juveniles were obtained from a fish farm (Futuna Blue, Cádiz, Spain), transported live to the Aquaculture Laboratory of Polytechnic University of Valencia and randomly distributed in experimental tanks.

Prior to the feeding trial, all fish were acclimatized to the indoor rearing conditions for 4 weeks and fed a standard diet (550 g/kg crude

protein, CP; 140 g/kg crude lipid, CL; 110 g/kg ash; 22 g/kg crude fibre, CF; and 140 g/kg nitrogen-free extract, NFE). Groups of 19 fish (average weight 38.4 ± 11.6 g) were housed in 12 cylindrical fibreglass tanks (three per treatment).

All fish were weighed every 30 days. Previously, fish were anaesthetized with 10 mg/L clove oil (Guinama®), containing 87 % eugenol. The fish were not fed for 1 day before weighing. At the beginning of the experimental trial, five fish were sampled and stored at −30°C for subsequent whole-body composition analyses. The fish were slaughtered by a thermo-shock in a melting ice bath.

At the end of the growth trial, all fish per tank were sampled to determine biometric parameters (viscerosomatic and hepatosomatic indices, condition factor and mesenteric fat) and three specimens per tank were randomly sampled and pooled to determine the proximate and amino acid body composition.

2.3 Diets and feeding

Four isolipidic (140 g/kg of CL) and isoenergetic diets (24 MJ/kg of gross energy, GE) were formulated (Table 1) with the same digestible protein (50 g/kg, DP), and with 530–633 g/kg of crude protein (CP). For diet formulation, individual ingredient digestibility coefficients were taken from a previous study (Tomás-Vidal et al., 2019) to estimate protein digestibility.

FM100 was used as the control diet with a high FM content (525 g/kg). In the FM66, FM33 and FM0 diets, fish meal was replaced by an alternative protein blend (corn gluten meal, krill meal and meat meal) at 33 g/kg, 66 g/kg and 100 g/kg, respectively. FM33 and FM0 diets were supplemented with synthetic L-Met and L-Lys in amounts of 3–5 g/kg, respectively, to simulate the digestible amino acid profile of the fish meal diet. (Tomás-Vidal et al., 2019). The composition of the experimental diets and their proximate values are shown in Table 5.1.

Table 5.1 Formulation and proximate composition of experimental diets.

	Diets			
	FM100	**FM66**	**FM33**	**FM0**
Ingredients (g/kg)				
Fish meal	525	350	175	
Wheat	235	108	43	
Wheat gluten	130	130	140	180
Corn gluten		100	100	100
Defatted krill		120	230	345
Meat meal		80	198	250
Fish oil	90	92	88	95
L- Methionine [a]			3	5
L-Lysine Clh [a]			3	5
Vitamin-mineral mix [b]	20	20	20	20
Proximate composition (g/kg dry matter)*				
Dry matter (DM)	888	888	895	902
Crude protein (CP)	530	580	604	633
Crude lipid (CL)	139	142	138	137
Ash	103	106	121	115
N-Free Extract (NFE) [c]	228	171	137	115
GE (MJ/kg) [d]	23.8	24.1	23.7	23.8
DP (g/kg) [e]	497	504	497	479
DE (MJ/kg) [e]	20.3	19.2	18.1	16.3
DP/DE (g/MJ) [g]	24.5	26.2	27.5	29.4

a L-Methionine and L-Lysine Clh: Guinama S.L.U.

b Vitamin and mineral mix (values are g/kg except those in parentheses): Premix: 25; Choline, 10; DL-a-tocopherol, 5; ascorbic acid, 5; $(PO_4)_2Ca_3$, 5. Premix composition: retinol acetate, 1 000 000 IU/kg; calcipherol, 500 IU/kg;

DL-a-tocopherol, 10; menadione sodium bisulphite, 0.8; thiamine hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamine, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol,15; betaine, 100; polypeptides 12. Zn, 5; Se, 0.02; I, 0,5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02; Met, 1.2; Cys, 0.8; Lys, 1.3; Arg, 0.6; Phe, 0.4; Trcp, 0.7; excpt. 1000 g.

* By analysis.

c NFE (Nitrogen-Free Extract) = 1000 - CP (g/kg) - CL (g/kg) - Ash (g/kg) - CF (g/kg).

d GE (MJ/kg) was calculated according to Brouwer from the C (g) and N (g) balance (GE = 51.8 × C − 19.4 × N). The C–N was analysed by the Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA).

e Digestible protein (DP), digestible energy (DE): were calculated based on the respective values of apparent digestibility coefficients (ADC) estimated by a digestibility trial: ADC protein (%) (diet 100 = 94; diet 66 = 87; diet 33 = 82; diet 0 = 76) and ADC energy (%) (diet 100 = 85.5; diet 66 = 79.5; diet 33 = 76.5; diet 0 = 68.5).

g DP/DE = DP (g/kg)/DE (MJ/kg).

Diets were prepared by cooking extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45). The processing conditions were as follows: 100 rpm speed screw, 110°C temperature, 40–50 atm pressure, producing pellets having a 3–5 mm diameter. Experimental diets were assayed in triplicate. Fish were fed by hand twice a day (9:00 and 17:00 p.m.) from Monday to Saturday until apparent satiation. Pellets were distributed slowly, allowing all fish to eat, and the total amount of feed distributed was recorded.

2.4 Proximate composition and amino acid analysis

Chemical analysis of the dietary ingredients was performed prior to diet formulation. Dietary ingredients, diets (Table 5.1) and the whole fish were analysed according to AOAC (1995) procedures: dry matter (drying at 105°C to constant weight), ash (incinerated at 550°C to constant weight) and crude protein by the Kjeldahl procedure (N × 6.25) after acid digestion (2300 Kjeltec Analyzer Unit) and crude lipids were extracted with diethyl ether (ANKOM XT10) by the Dumas principle. The energy was calculated according to Brouwer, (1965), from the C (g) and N (g) balance (GE = 51.8 × C − 19.4 × N). Carbon and nitrogen were analysed by the Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA).

Following the method previously described by Bosch et al., (2006), the amino acids of the diets (Table 5.2), fish carcasses and faeces were analysed on a Waters HPLC system consisting in two pumps (Model 515; Waters), an autosampler (Model 717; Waters), a fluorescence detector (Model 474; Waters) and a temperature control module. Aminobutyric acid was added as an internal standard after hydrolysation. Amino acids were derivatized with AQC (6-aminoquino lyl-N-hydroxysuccinimidyl carbamate). Methionine and cysteine were determined separately as methionine sulphone and cysteine acid after oxidation with performic acid. Amino acids were separated by a C-18 reverse-phase column Waters AcQ. Tag (150 mm × 3.9 mm). All analyses were performed in triplicate, except for faeces analysis, which was performed in duplicate.

2.5 Digestibility and retention estimations

Simultaneously with the feeding trial, 16 fish with the same weight as the experiment were used in a trial designed to determine the apparent digestibility of the experimental diets. The digestibility system was constructed according to the Guelph System protocol (Cho et al., 1982), using four digestibility tanks (4 fish/tank). The water temperature averaged 20.5 ± 2.1°C, all tanks were equipped with aeration, and the level of dissolved oxygen was 6.6 ± 1.3 mg/L. The photoperiod was maintained at 12-hr light and 12-hr dark by means of artificial daylight simulation. The same four diets were used, but chromium oxide (5 g/kg) was added as an inert marker.

Table 5.2 Amino acids composition of experimental diets.

	Experimental diets			
	FM100	**FM66**	**FM33**	**FM0**
EAA (g/kg in dry matter)				
ARG	35.1	31.9	37.0	34.5
HIS	11.8	12.6	11.7	12.3
ILE	25.8	27.4	27.1	26.7
LEU	42.9	52.8	51.6	52.7
LYS	33.3	29.2	32.1	28.0
MET	11.0	9.9	11.6	12.5
PHE	25.8	26.9	26.8	28.3
THR	22.0	19.7	20.5	21.1
VAL	31.9	32.1	32.8	33.1
*Cyst**	4.9	5.0	4.9	7.0
*Tyr**	15.4	17.9	18.6	19.3
NEAA (g/kg in dry matter)				
Ala	29.9	33.3	34.8	33.7
Asp	42.9	46.4	49.8	45.4
Glu	109.1	129.5	126.8	146.2
Gly	29.4	33.0	35.1	38.8
Pro	32.2	39.5	39.7	46.9
Ser	20.3	21.4	22.4	25.7
EAA/NEAA	0.99	0.88	0.89	0.82

EAA, Essential amino acids; NEAA, Non-essential amino acids. **Cys* and *Tyr* are cosiderated Semi-essential amino acids.

The fish were fed at 10.00 hr. The feed was offered only, while the fish were actively feeding to avoid wastage. One hour after the meal, the drainpipe and the settling column were brushed out to remove uneaten feed particles from the system. Faeces were collected from the base of the settling column into a plastic container by gravity 8 hr after the feeding. The fish were fed again at 10.00 hr just as every day.

Latin square design with four diets and four periods per square. Each period lasted 30–35 days for each diet and each replicate, the last 3 days of the period fish fasted to avoid mixing faeces between diets. Wet faecal content was collected and freeze-dried prior to analysis. Chromium oxide was determined in the diets and the faeces using an atomic absorption spectrometer (Perkin Elmer 3300) after acid digestion. On two of the diets, two replicas suffered a problem in the analysis and were discarded not to alter the data.

The apparent digestibility coefficients (ADCs) for protein, energy, dry matter and amino acids of the diets tested were calculated with the following formula:

$$\text{ADC}\ (\%) = \ 100 \text{ x } \left[1 - \left(\frac{\text{F}}{\text{D}} \text{ x } \frac{\text{DCr}}{\text{FCr}}\right)\right]$$

where F is the percentage of nutrient or energy in faeces, D is the percentage of nutrient or energy in the diet, DCr is the percentage of chromic oxide in the diet, and FCr is the percentage of chromic oxide in faeces (Cho & Kaushik, 1990).

Protein, energy and AA retention efficiencies were calculated.

2.6 Statistical analysis

Results from growth data and nutrient retention parameters were treated using multifactor analysis of variance (ANOVA), introducing the initial live weight as covariate (Snedecor & Cochran, 1971) to assess the final weight. Digestibility and nutrient retention data were treated using a one-way ANOVA. The Newman–Keuls test was used to assess specific differences among individual diets at 0.05 significant level (Statgraphics, Statistical Graphics System, Version Plus 5.1).

2.7 Ethical statement

The *Seriola dumerili* study complied with European Union Council Directive 2010/63/UE, which lays down minimum standards for the protection of animals, and was also in accordance with Spanish national legislation (Spanish Royal Decree 53/2013) protecting animals used in experimentation and for other scientific purposes. The experimental protocol was approved by the Ethics Committee of Polytechnic University of Valencia (UPV).

Fish in the tanks were checked daily. Additionally, fish were weighed individually every 4 weeks and their health status was assessed through observation after sedation with clove oil disolved in water (0.01 mg/L of water) to minimize animal suffering. Animals were euthanized by an excess of clove oil (150 mg/L) and then dissected.

3. RESULTS

Survival of fish at day 119 of the experiment was approximately 90%, but was negatively affected at day 154 due to a non-specific fish disease causing high mortality (Figure 5.1). Fish fed the FM0 diet exhibited the lowest survival rate (23%), while fish fed the FM100, FM66 and FM33 diets presented similar survival rates (from 75% to 86%).

All experimental fish groups grew from the beginning of the trial and, at the end of the experiment, no differences in growth were obtained, as shown in Table 5.3. Although no significant differences were found, a linear regression was performed to determine whether there was indeed a trend between FM substitution and growth and a clear tendency was observed (R^2 = .835).

For to FI, DPI, DEI, FCR and PER, no significant differences were observed among the diets.

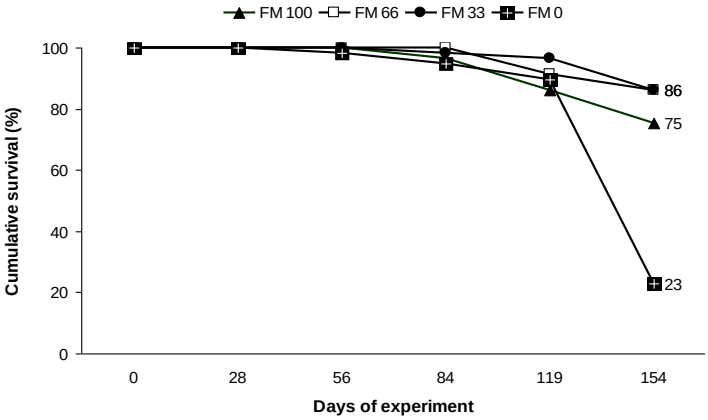


Figure 5.1 Evolution of survival of the greater amberjack with the experimental diets.

Table 5.3 Effect of the different diets on growth and feed utilization efficiency in *Seriola dumerili*.

Parameters	FM100	FM66	FM33	FM0	P-value
Initial weight (g)	39.6 ± 1.5	38.1 ± 1.5	38.1 ± 1.5	36.7 ± 1.5	0.6031
Survival (%)	75[a] ± 6	86[a] ± 6	86[a] ± 6	23[b] ± 6	0.0002
Final weight (g)	385 ± 22	391 ± 22	348 ± 22	333 ± 22	0.2799
SGR (%/day) [a]	1.50 ± 0.04	1.51 ± 0.04	1.43 ± 0.04	1.40 ± 0.04	0.2571
FI (g100 g/fish day) [b]	1.83 ± 0.10	1.77 ± 0.10	1.81 ± 0.10	1.77 ± 0.10	0.9506
DPI (g 100 g/fish day) [c]	0.80 ± 0.04	0.79 ± 0.04	0.80 ± 0.04	0.78 ± 0.04	0.9632
DEI (kJ 100 g/fish day) [d]	33.0 ± 1.5	30.2 ± 1.5	29.4 ± 1.5	26.6 ± 1.5	0.1015
FCR [e]	1.77 ± 0.11	1.68 ± 0.11	1.74 ± 0.11	1.80 ± 0.11	0.9062
PER [f]	1.20 ± 0.06	1.16 ± 0.06	1.06 ± 0.06	0.98 ± 0.06	0.1735

Means of triplicate groups. Values are presented as mean ± SEM (standard error of the pooled means). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

Initial weight was considered as covariable for final weight and SGR.

[a] Specific growth rate (% day $^{-1}$) SGR = 100 x ln (final weight-initial weight)/days.

[b] Feed intake (g 100 g fish $^{-1}$ day $^{-1}$) FI = 100 x feed consumption (g)/average biomass (g) x days.

[c] Digestive protein intake DPI = 100 x digestive protein consumption (g)/average biomass (g) x days.

[d] Digestive energy intake DEI = 100 x digestive energy consumption (kJ)/average biomass (g) x days.

[e] Feed conversion ratio FCR = feed offered (g)/weight gain (g).

[f] Protein efficiency ratio PER = biomass gain (g)/protein intake (g).

The digestible protein and energy intake were lower in fish fed the FM0 diet (7.76 gr/kg day/266 kJ/kg fish day) than in fish fed the FM100 and FM66 diets ADC (%) = 100×[1−(F⁄D×DCr⁄FCr)] (8.03 and 7.90 g kg/fish day / 330 and 302 kJ/kg fish day, respectively) but without significant differences.

Concerning biometric parameters (Table 5.4), significant differences were observed in the viscerosomatic index (VSI), which showed its highest value in fish fed the FM0 diet (5.87%). Hepatosomatic index (HSI) and mesenteric fat index (MF) did not present any significant differences among diets.

In terms of whole-body composition, significant differences were found in protein content. Fish fed the control diet (FM100) showed the highest value (192.2 g/kg) and fish fed the FM0 diet presented the lowest (177.7 g/kg). The moisture, lipid and ash contents were not affected by the level of fish meal substitution.

Only ingested protein retention efficiency showed significant differences (IPR, %). The value of fish fed the FM0 diet (17.5%) was significantly lower than that related to the FM100 and FM66 diets (23.7% and 21.9%, respectively), but no differences were found for the FM33 diet. In general, the FM0 diet exhibited lower retention values than the other diets except in DER (%) which showed the inverse trend.

Regarding the dietary EAA level (Table 5.2), a higher level of Leu was observed in experimental diets than in FM100 diet (52.8 and 51.6 g/kg in FM33 and FM66, respectively). The level of other dietary EAAs was similar among experimental diets except in Lys, where FM66 and FM0 diets showed the lowest Lys content (29.2 and 28.0 g/kg, respectively), although the latter had been supplemented with synthetic L-Lys. However, the dietary Met level in FM0 diet was higher than FM100 (12.5 g/kg and 11 g/kg, respectively). Moreover, the EAA/NEAA ratio was lower in the FM0 diet than in the FM100 diet (0.82 versus 0.99) because of the increased NEAA dietary levels, which are higher as dietary fish meal substitution increases.

Table 5.4 Biometric indices, whole-body composition and retention efficiency of *Seriola dumerili* at the end of the experiment.

Parameters	Initial	FM100	FM66	FM33	FM0	P-value
CF [a]		1.37 ± 0.06	1.44 ± 0.06	1.47 ± 0.06	1.48 ± 0.06	0.6349
MF(%) [b]		0.18 ± 0.05	0.14 ± 0.05	0.10 ± 0.05	0.04 ± 0.06	0.3263
HSI(%) [c]		0.87 ± 0.10	1.05 ± 0.10	1.02 ± 0.10	1.22 ± 0.12	0.1950
VSI(%) [d]		4.32^{c} ± 0.23	4.87^{bc}±0.23	5.15^{b} ± 0.23	5.87^{a} ± 0.24	0.0006
Moisture (g/kg)	768.0	696.5 ± 3.3	701.8 ± 3.3	704.1 ± 3.3	709.1 ± 3.3	0.0772
CP (g/kg ww)	164.6	192.2^{a} ± 1.5	186.1^{b} ± 1.5	188.0^{ab} ± 1.5	177.7^{c} ± 1.5	0.0000
CL (g/kg ww)	30.6	77.8 ± 2.7	75.5 ± 2.7	72.9 ± 2.7	78.4 ± 2.7	0.4767
Ash (g/kg ww)	36.7	28.1 ± 2.0	31.7 ± 1.9	30.3 ± 1.9	28.4 ± 1.9	0.5261
Ingested values						
IPR(%) [e]		23.7^{a} ± 1.2	21.9^{a} ± 1.2	20.4^{ab} ± 1.2	17.5^{b} ± 1.2	0.0393
IER(%) [f]		21.7 ± 1.3	21.8 ± 1.3	21.1 ± 1.3	20.3 ± 1.3	0.8294
Digested values						
DPR(%) [g]		25.3 ± 1.5	25.2 ± 1.5	24.8 ± 1.5	23.2 ± 1.5	0.7400
DER(%) [h]		25.3 ± 1.8	27.5 ± 1.8	27.5 ± 1.8	29.6 ± 1.8	0.4645

Means of triplicate groups. Values are presented as mean ± SEM (standard error of the pooled means). Values in the same row having different superscript letters are significantly different ($P < 0.05$).

[a] Condition factor CF = 100 x total weight (g)/total length^3 (cm).

[b] Mesenteric fat (%) MF = 100 x mesenteric fat weight (g)/fish weight (g).

[c] Hepatosomatic index (%) HSI = 100 x liver weight (g)/fish weight (g).

[d] Viscerosomatic index (%) VSI = 100 x visceral weight (g)/fish weight (g).

[e] Ingested protein retention (%) IPR = 100 x fish protein gain (g)/crude protein intake (g).

[f] Ingested energy retention (%) IER = 100 x fish energy gain (kJ)/gross energy intake (kJ).

[g] Digestible protein retention (%) DPR = 100 x fish protein gain (g)/ digestible protein intake (g).

[h] Digestible energy retention (%) DER = 100 x fish energy gain (kJ)/digestible energy intake (kJ).

Regarding the ADC coefficients (Table 5.5), no differences were found in ADC for the dry matter. However, there are significant differences in the protein ADC, which diminished according to fish meal substitution, so the FM0 diet obtained a lower value (75.7%) than the FM100 and FM66 diets (93.7% and 86.8%, respectively). Likewise, the energy ADC coefficient in the FM0 diet was the lowest (68.5%), followed by the FM33 diet (76.5%).

The same tendency observed in the ADC for protein was shown in essential amino acids (EAA ADC_{AA}). In general, considering only the EAAs, ADC_{AA} increased with the fish meal dietary content, and only the Met, Val and Thr did not show any significant differences. Lys presented the lowest digestibility in the FM0 diet (69.4 %). For NEAA, the ADC_{AA} was affected with significant differences in the case of Ala, Cys, Glu, Pro and Tyr. Therefore, in digestible terms, the relation EAA/NEAA diminished according to FM dietary substitution increases.

Figure 5.2 shows the digestible EAA intake (g/AA kg of fish day, DAA). ADC_{AA} had a strong influence particularly on the Lys intake, which became significantly higher in fish fed the control diet (FM100) than in those fed the FM0 diet. Furthermore, no significant differences were detected between the rest of the AAs and dietary intake between diets.

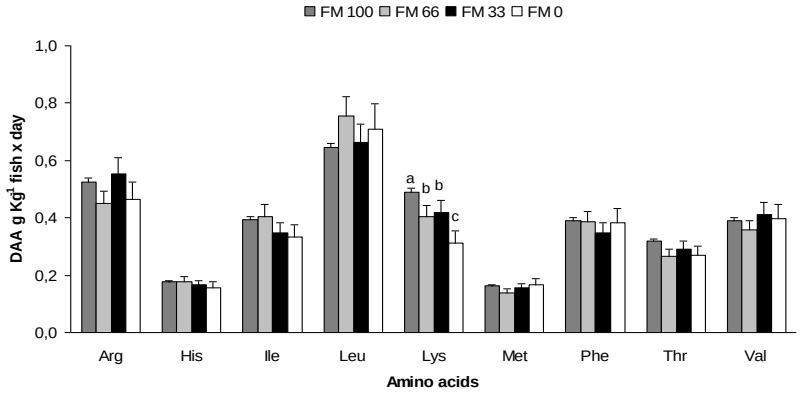


Figure 5.2 Digestible essential amino acids intake in each experimental diet, expressed as g/Kg of fish per day.

At the end of the trial, the fish fed experimental diets showed significant differences in the Arg, and Gly levels of the entire fish body (Table 5.6). Fish fed the FM100 and FM0 diets showed the highest and the lowest values, respectively, but no significant differences were observed between fish fed the FM66 and FM33 diets. The EAA/NEAA ratio of the whole body was similar among all the experimental diets.

The amino acid retention efficiency (%) of ingested (a) and digested (b) EAA in fish fed the experimental diets at the end of the experiment is shown in Figure 5.3a, b, respectively. Without considering the effect of the diet, in ingested AA, His and Lys retention efficiency showed the highest efficiency values (27.83% and 33.14%), while Leu and Phe retention efficiency presented the lowest values (18.88% and 18.12%, respectively). Concerning the retention efficiency of EAA ingested (%), fish fed the FM0 diet showed lower Met retention (19.79%) than fish fed the FM100 and FM66 diets (26.16% and 30.25%, respectively). In the retention efficiency of EAA digested, fish fed the FM0 diet exhibited the highest Lys retention efficiency (50.99%) and fish fed the FM100 diet, the lowest (30.94%). The retention efficiency of digested Met resulted in higher levels in fish fed the FM66 diet (33.83%) than in fish fed the FM33 and FM0 diets (27.42% and 24.03%, respectively).

The AA index, or ratio between diet and whole-fish body EAA profile, is shown in Figure 5.4. In the present experiment, significant differences were observed in the AA index for Arg, Ile, Leu, Lys, Met, Thr and Val (Figure 5.4). Fish fed the FM0 diet exhibiting an AA index below that of 100, except for Leu and Phe, and fish fed the FM100 diet, presented values higher than 100 for Ile, Leu and Phe.

In the AA index of Lys, a decreasing tendency can also be appreciated as the dietary level of fish meal substitution increased, more specifically, showing a drastic decrease and presenting the lowest values in fish fed the FM0 diet (45.43%).

Table 5.5 Apparent digestibility coefficients (ADCs) of experimental diets.

	Diets				
ADCs (%)	**FM100 (n=4)**	**FM66 (n=2)**	**FM33 (n=2)**	**FM0 (n=4)**	**P-value**
Dry mater	67.5 ± 1.8	63.3 ± 2.9	58.6 ± 2.9	63.8 ± 2.0	0.1349
Protein	93.7^{a} ± 1.9	86.8^{ab} ± 3.1	82.2^{bc} ± 3.1	75.7^{c} ± 2.2	0.0012
Energy	85.5^{a} ± 4.1	79.5^{ab} ± 4.1	76.5^{ab} ± 4.1	68.5^{b} ± 2.9	0.004
EAA					
ADC_{AA}					
ARG	92.8^{a} ± 1.8	90.2^{ab} ± 2.8	92.7^{a} ± 2.8	83.6^{b} ± 2.0	0.0301
HIS	93.6^{a} ± 2.2	90.0^{a} ± 2.7	87.7^{a} ± 2.7	78.5^{b} ± 1.9	0.0066
ILE	95.0^{a} ± 2.2	90.4^{a} ± 3.1	79.7^{b} ± 3.1	77.4^{b} ± 2.2	0.0022
LEU	93.1^{a} ± 2.0	90.7^{ab} ± 2.9	79.5^{c} ± 2.9	83.5^{bc} ± 2.0	0.0136
LYS	91.1^{a} ± 3.1	88.4^{a} ± 4.4	79.7^{b} ± 4.4	69.4^{c} ± 3.1	0.0060
MET	91.7 ± 2.3	89.4 ± 3.2	82.6 ± 3.2	82.4 ± 2.3	0.0654
PHE	93.8^{a} ± 1.8	91.5^{a} ± 2.6	80.5^{b} ± 2.6	84.2^{b} ± 1.8	0.0078
THR	89.5 ± 2.4	85.8 ± 3.4	88.1 ± 3.4	79.0 ± 2.8	0.1064
VAL	76.0 ± 4.9	71.0 ± 6.9	77.8 ± 6.9	74.5 ± 5.7	0.9042
*Cys**	87.3^{a} ± 2.0	85.4^{ab} ± 3.2	78.3^{bc} ± 3.2	75.2^{c} ± 2.6	0.0246
*Tyr**	78.2^{a} ± 3.3	77.3^{ab} ± 4.7	62.3^{bc} ± 4.7	48.8^{c} ± 4.7	0.0087
NEAA					
ADC_{AA}					
Ala	90.6^{a} ± 2.7	88.1^{ab} ± 3.8	75.9^{b} ± 3.8	79.8^{b} ± 2.7	0.0332
Asp	85.9 ± 2.4	85.3 ± 3.9	82.3 ± 3.9	74.9 ± 2.7	0.0683
Glu	95.1^{a} ± 1.8	92.8^{a} ± 2.6	90.0^{a} ± 2.6	82.0^{b} ± 1.8	0.0052
Gly	90.1 ± 2.7	85.7 ± 3.8	83.6 ± 3.8	77.2 ± 2.7	0.0542
Pro	94.2^{a} ± 2.2	91.5^{a} ± 2.7	91.3^{a} ± 2.7	82.8^{b} ± 1.9	0.0251
Ser	88.8 ± 3.0	84.1 ± 3.7	86.8 ± 3.7	77.5 ± 2.6	0.1032

Values are presented as mean ± SEM (standard error of the pooled means). Values in the same row having different superscript letters are significantly different ($P < 0.05$).

EAA, Essential amino acids; NEAA, Non-essential amino acids. **Cys* and *Tyr* are cosiderated as semi-essential amino acids.

Digestible amino acids were determined by faeces analysis; ADC_{AA} (%) = 100 x [1- (Cr_2O_3 in diet/ Cr_2O_3 in faeces) x (AA in faeces/AA in diet)]. Values in the same row having different superscript letters are significantly different ($P < 0.05$).

Table 5.6 Effects of diets on whole-body amino acid composition at the end of the trial.

Parameters	Initials	FM100	FM66	FM33	FM0	SEM	P-value
EAA (g/kg ww)							
ARG	11.8	14.8^{a}	13.5^{ab}	13.9^{a}	12.3^{b}	0.41	0.0148
HIS	3.9	5.5	4.7	5.2	4.8	0.23	0.0999
ILE	7.2	8.6	8.8	9.2	9.4	0.47	0.6239
LEU	12.1	13.6	14.4	14.7	14.4	0.46	0.4372
LYS	13.2	14.5	15.2	16.2	15.6	0.45	0.1215
MET	4.9	4.5	4.5	4.2	4.1	0.16	0.2031
PHE	6.1	7.5	7.3	7.5	7.3	0.35	0.9477
THR	7.1	8.6	8.3	8.5	7.8	0.33	0.3322
VAL	9.2	10.3	10.4	11.1	10.7	0.34	0.3456
*Cys**	2.1	2.1	1.7	1.5	1.7	0.14	0.1072
*Tyr**	4.5	4.9	4.6	5.4	4.2	0.58	0.5623
NEAA (g/kg ww)							
Ala	10.8	12.7	12.3	12.3	12.0	0.19	0.1434
Asp	15.4	17.6	17.3	17.9	17.9	0.45	0.8023
Glu	24.1	28.5	29.0	29.4	28.7	0.53	0.6584
Gly	12.5	17.5^{a}	14.2^{b}	13.7^{b}	13.5^{b}	0.55	0.0025
Pro	7.3	9.2	8.7	8.1	7.8	0.56	0.3118
Ser	6.6	7.7	7.5	7.2	6.7	0.23	0.0572
EAA/NEAA	1.07	1.02	1.05	1.10	1.07	0.04	0.4699

Data in the same row with different superscripts differ at $P < 0.05$. Dates are the mean of triplicate group ± SEM (standard error of the pooled means).
EAA, Essential amino acids; NEAA, Non-essential amino acids. *Cys and Tyr are cosiderated as semi-essential amino acids.

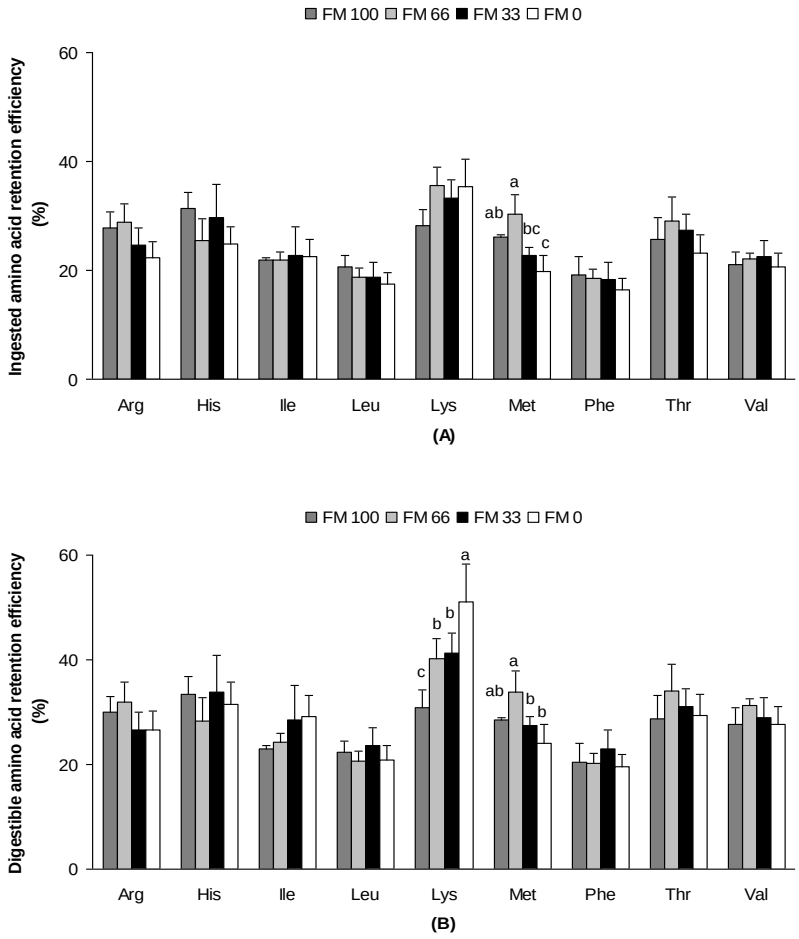
4. DISCUSSION

The fish presented a satisfactory growth throughout the trial. The TGC average obtained in the present experiment was higher than that obtained in previous studies in *S. dumerili* (Jover et al., 1999; Tomás-Vidal et al., 2008; Tomás et al., 2005). The TGC value in this case was not significantly different between diets but was fairly higher than that of other Mediterranean aquaculture species such as *Sparus aurata* (1.78×10^{-3}*), Argyrosomus regius* (3×10^{-3}), *Sciaenops ocellatus*

(between 0.5 and 1×10^{-3}) or *Seriola dumerili* (1×10^{-3}) (Mayer et al., 2008; Tomás et al., 2005; Velazco-Vargas et al., 2013), which demonstrates that *S. dumerili* is a high growth specie and is a good alternative to aquaculture diversification, due to the possibility of using the same facilities and obtaining better results.

Although no significant differences were found in the growth of fish fed experimental diets, a negative tendency ($p > .05$) was observed when fish meal substitution was increased. This fact is in accordance with the results obtained in several studies in which soybean meal was used at the highest dietary levels as a partial substitute for fish meal (Dawood et al., 2015; Tomás et al., 2005) or poultry by-product meal (Takakuwa et al., 2006a). It also agrees with studies on *S. quinqueradiata*, in which the fish meal content in diets can be reduced to approximately 300 g/kg diet using alternative protein sources (Aoki et al., 2000; Watanabe et al., 1994). However, further replacement of fish meal by alternative proteins results in inferior growth and feed utilization, as well as the development of abnormal physiological conditions, such as anaemia and a higher incidence of green liver originated by biliverdin pigment (Maita & Aoki, 1997). Taurine deficiency is the main reason for the outbreak of Green liver in Japanese yellowtail (Takagi et al., 2006) and in red sea bream (Goto et al., 2001). In our experiment, green liver was not detected probably because the diet contains a high Tau concentration supplied to the diets by defatted krill. Taurine is in high concentration in animal tissues. Consequently, the removal of taurine-rich dietary ingredients such as fish meal (3,200 mg Tau/kg) may create a deficiency (Salze & Davis, 2015; Spitze et al., 2003) so the addition of animal meals such as krill meal (2,890 mg Tau/kg) and meat meal (1,150 mg Tau/kg) could reduce this deficiency (Chi et al., 2013).

The low hepatic taurine level is the main reason for the Green liver, with the negative consequence on bile pigment metabolism. Therefore, the addition of taurine to diets is recommended to avoid this abnormality.



Significant differences (P < 0.05) are indicated with different letters.
Amino acid retention efficiency (AARE) (%) = 100 x fish AA gain (g)/ingested AA (g).
Digestible amino acid retention efficiency (DAARE) (%) = 100 x fish AA gain (g)/digested AA (g).

Figure 5.3 Amino acid retention efficiency (%) of ingested and digested essential amino acids in *Seriola dumerili* fed with the experimental diets at the end of the experiment (mean ± SD, n = 3).

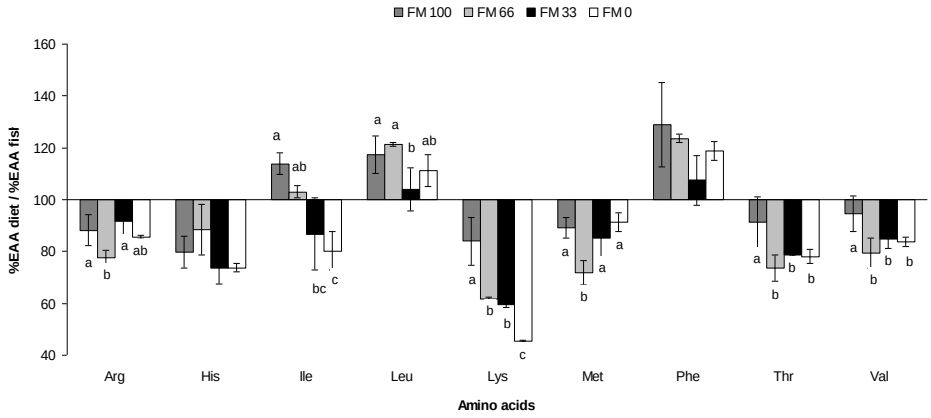


Figure 5.4 Ratio between the essential digestible amino acid profile of experimental diets and whole fish body. Each value is the mean ± SD of triplicate groups. Different superscripts indicated differ at $P < 0.05$.

The main problem of total fish meal replacement by alternative protein sources in this experiment was the high mortality observed during the last 30 days, with the death of 75% of the fish fed that diet. The survival of fish fed the FM0 diet before this episode was fish fed a non-fish meal diet initially fed actively and grew normally, but afterwards growth stagnated, and high mortality was observed due to a bacterial infection (Maita et al., 1998). In addition, fish fed a non-fish meal diet exhibited anaemia and hypocholesterolaemia (Dawood et al., 2015; Maita et al., 1998, 2006; Maita & Aoki, 1997). The susceptibility to opportunistic infections due to the weakening of the fish immune system has also been observed in other species (Estruch et al., 2015) when fed without dietary fish meal.

This weakening of the immune system can also be explained by the lack of nutrient availability (there is a very clear decrease in most of the amino acids and energy digestibility of the FM0 diet) caused by some antinutrient factors contained, not only in vegetable meals, but also in krill by-product. In the case of krill, arthropods are usually poor in carbohydrates, but they contain chitin, composed of an unbranched polymer of N-acetylglucosamine that might reduce the access of chitinases or proteinases to their substrates and prevent protein and

lipid absorption in the intestine, producing a low nutrient efficiency and decreasing growth. In our experiment, lipid digestibility could not be carried out, but the detriment of energy digestibility, coinciding with the dietary defatted krill meal increase, would support this fact. The low DEI on the FM0 diet could also be related to the low intake of digestible energy, which in addition could not have been enough to maintain fat deposits as in other diets, despite the fact that growth had been maintained. However, the reduction in nutrient digestibility in fish depends on the species: poor nutrient digestibility was observed in rainbow trout fed 25 g/kg chitin and *Salmo salar* fed with 5 g/kg of dietary chitin (Karlsen et al., 2017; Lindsay et al., 1984). Nevertheless, *Gadus morhua* or Atlantic cod, in which a decrease in nutrient digestibility was not observed, is able to digest and utilize the chitin. Another problem associated with the high content of krill meal is that the dietary content of fluoride derived from Antarctic krill could also affect the digestibility and inhibit fish growth (Yoshitomi & Nagano, 2012).

The relatively low ADCs of energy obtained in fish fed the FM0 diet can be attributed to several factors: the high content of chitin, non-digestible carbohydrates (Aslaksen et al., 2007) and the high fibre content from krill, and the high ash content from meat meal. All of those factors may increase intestinal transit and reduces gut-retention time of feed and time available for nutrient digestion (Fountoulaki et al., 2005). Additionally, the presence of chitin from krill meal and its negative influence on lipid digestibility (Kroeckel et al., 2012) could affect the energy digested by fish fed a diet with a high content of krill meal, as in the FM0 diet. The detriment of digestible energy intake was a consequence of the ADC energy coefficients presented in this diet, and therefore, the efficiency retention showed a clear tendency to increase when fish meal substitution increased and, although significant differences were not observed, fish fed the FM0 diet showed an energetic deficiency. Meat meal also affected the protein ADC of diets and contributed (alone or synergistically with krill) to the lower performances observed.

Lys is one of the main limiting amino acids (Gatlin et al., 2007). Its dietary imbalance could be the main reason for fish mortality, also

observed in Midas (*Amphilophus citrinellus*) by Dabrowski et al., (2007). A possible justification of the lower Lys digestibility presented in this trial can be related to the animal meal (particularly meat meal) included at high levels in the FM0 diet. The excessive heat applied during its production might damage proteins, especially affecting Lys (Carpenter & Booth, 1973; Opstvedt et al., 1984), which may contribute to lower protein digestibility. In addition, the protein source (muscle, connective tissue, bones, etc.) also affects digestibility. In this sense, Allan et al., (2000) observed a lower Lys digestibility coefficient in meal obtained from bones than in fish meal. Additionally, EAA digestibility diminishes with increasing the dietary substitution of FM, and therefore, the lower EAA/NEAA ratio could lead to inferior growth and reduce protein retention (Marcouli et al., 2004; Masumoto et al., 1996; Yamamoto et al., 1998). Vegetable meals contain indigestiblecomponents, as well as protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins and allergens (Francis et al., 2001), that cause low protein availability, histological gut alteration and an unbalanced microbiota (Estruch et al., 2015) and that may alter the immune regulatory functions of the gut and contribute to the development of diseases (Pérez et al., 2010). Yellowtail kingfish (*Seriola lalandi*) fed with solvent-extracted soybean meal and soy protein concéntrate produced a development of subacute enteritis in the hindgut that may compromise fish health upon long-term feeding (Bansemer et al., 2015). In this experiment, the vegetal protein content was smaller due to the substitution of animal protein and that could be a reason why all the described effects can be diminished.

Overall, the low EAA digestibility of FM0 diet caused the lowest intake of digested EAA of fish fed with this diet. However, only digested Lys efficiency retention of fish fed the FM0 diet exhibited the highest values indicating that Lys is the limiting amino acid for protein synthesis in fish fed the FM0 diet.

The amino acid index is the result of the ratio between the EAA profile in experimental diets and the whole-body fish at the end of the trial. When this index is below 100, it might indicate that the AA is in minor proportion compared to in the ideal protein in the diet and, as a

consequence, it would have a high retention. Nevertheless, if the AA index is higher than 100, this AA could be in higher % in the diet. Therefore, the whole-body efficiency retention would be low (Sánchez-Lozano et al., 2011). Moreover, the Lys amino acid index in the present experiment corroborates that the percentage of digestible Lys in the FM0 diet is significantly lower than in the other diets and did not cover the yellowtail Lys requirements. Therefore, Lys should be in a higher percentage at least in FM0.

In summary, we can conclude from the results of this experiment that the total fish meal replacement by the alternative protein blend assayed was not feasible for yellowtail feeding, causing a detriment of digestible EAA and energy upon long-term feeding and high mortality due to the poor immune response to opportunistic pathogens. Fish meal substitution at the 66 g/kg dietary level obtained good growth and nutrient efficiency and high survival.

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Capítulo 6
Growth, sensory and chemical characterization of Mediterranean yellowtail (*Seriola dumerili*) fed diets with partial replacement of fish meal by other protein sources.

Aceptado (1/09/2020): Aquaculture Reports

ABSTRACT

An 84-day trial was performed to assess the use of alternative protein sources in *Seriola dumerili*. Three diets were used, FM100 diet, as a control diet without fish meal substitution, and FM66 and FM33 diets with a fish meal replacement of 330 g/kg and 660 g/kg, respectively. At the end of experiment, fish fed the FM33 diet showed the lowest growth, lowest protein efficiency ratio and lowest lipid retention efficiency; For fatty acid composition, there was a very significant decrease in most of the fatty acids between fish fed FM33 diet and the other two experimental diets. Heavy metals present some differences but are always lower than risk levels. In sensory analysis, differences between diets appeared in pH and color, and also in some texture parameters between FM33 and the other two diets. No differences appeared between diets related to flavor. In summary, long periods of feeding with high fish meal substitution in diets, affects negatively to *Seriola dumerili* growth; despite this the quality of the fillet was not affected even with a 66% of substitution.

Keywords: *Seriola dumerili*, fish meal, fatty acids, heavy metals, sensory análisis.

1. INTRODUCTION

With the growth of aquaculture, the global availability of fish meal (FM) and fish oil is decreasing. Nowadays, a great effort is being made to choose and develop diets where these marine ingredients can be totally or, at least, partially substituted by other plant or animal protein sources. It is now consensual that animal and plant protein and lipid sources are valid ingredients for fish feeding, but their inclusion in diets may have adverse effects on fish growth and fish quality. The alternative sources of proteins in carnivorous fish can produce some problems like low palatability, deficiency in amino acids, presence of anti-nutritional factors (Francis et al., 2001). or modifications in tissue fatty acid composition or free amino acids that could compromise the nutritional quality of flesh for human consumption.

Mediterranean yellowtail (*Seriola dumerili*) is a carangid, carnivorous fish, with a fast growth rate and an excellent flesh quality and market price (Nakada, 2000), its excellent biological characteristics make it an object of interest for international production. For these reasons, it is a great candidate for diversification of marine aquaculture, taking into account hat this species can improve the competitiveness of aquaculture companies.

On the other hand, *Seriola dumerili* requires high dietary protein levels, therefore its sustainable production needs the substitution of FM by other cheaper and more available ingredients. Nevertheless, studies carried out on the effects of alternative protein sources in diets for the Mediterranean yellowtail (*Seriola dumerili*) studies are still scarce; Tomás et al. (2005) and Dawood et al. (2015), which examined the availability of soybean meal; Takakuwa et al. (2006), researching into poultry by-product meal; Monge-Ortiz et al. (2018a) with a blend of animal and vegetal proteins in juveniles.

Due to the great future expected for *S. dumerili* in Mediterranean aquaculture, itis very important not only to stablish the optimum maximum FM replacement for zootechnical parameters, but also for meat quality, especially at commercial weight (around 1 kg).

There are many factors that can affect fish quality, but the most important of them is the diet composition used during the on-growing period. This will influence nutritional composition (protein and lipid level, fatty acid profile), fillet safety (absence of dangerous bacteria, parasites or chemical compounds) and organoleptic properties.

Due to FM contains between 7-10% of total lipids with high content of n3 PUFAS, the FM substitution alters the fatty acid profile of muscle, as it has been reported in other previous studies in *Seriola dumerili* (Monge-Ortiz et al. 2018b), or in Sparus aurata (De Francesco et al. 2004).

Regarding safety (absence of dangerous bacteria, parasites or chemical compounds) one of the most studied aspects in fish concerns the presence of contaminants, especially heavy metals. Some of the heavy metals are essential for human life at low concentrations and are present in seafood; but they can be toxic in high concentrations. In order to evaluate the possible risks of fish consumption for human health is important to determine the concentrations of heavy metals in commercial fish (Oehlenschlager, 2002) those most commonly associated with poisoning are arsenic, cadmium, chromium, lead and mercury.

Lastly, diet affects the freshness and the sensorial properties of the fillet. These can be summarized into color, taste, structure, texture, stability, odor, appearance and acceptability (Álvarez et al., 2008; Izquierdo et al. 2005; Regost et al., 2003; Tortensen et al., 2005; Turchini et al., 2007).Similar quality studies have been conducted in other species: such as in *Oncorhynchus mykiss*, De Francesco et al. (2004). *Salmo salar*, Bjerkeng (1997), *Senegalese sole*, Valente (2011), tested different levels of fish meal (FM) substitution by plant proteins, no finding relevant differences in sensorial properties. However, from the best of our knowledge this is the first study that analyse the fillet sensorial alterations in *Seriola dumerili* fed with alternative proteins.

For all these reasons, the objective of the present work was to study the growth of *Seriola dumerili* fed with different FM substitution levels, and its effect on the muscle fatty acid composition, the level of heavy metals, and the sensorial and organoleptic analysis of *Seriola dumerili* fillets.

2. MATERIAL AND METHODS

2.1. Fish and rearing conditions.

Seriola dumerili juveniles from a previous experiment carried out in the facilities of Universitat Politècnica de València (Monge-Ortiz et al., 2018a) being continuously fed with the same diets with the objective to grow them from commercial weight.

The trial was conducted in octagonal concrete tanks (3500 L) inside a recirculated seawater system at the aquaculture laboratory of Animal Science Department at the Universitat Politècnica de València, (Valencia, Spain). The tanks were set up in a marine water recirculation system (capacity 75 m^3) with a rotary mechanic filter and a gravity bio-filter with a capacity of around 6 m^3. All tanks were equipped with aeration and water was heated by a heat pump installed in the system (TRANE CAN 490, 123.3 kW). The equipment used to control water parameters were an oxy-meter (OxyGuard, Handy Polaris V 1.26), a refractometer with 0 - 100 g/L range (Zuzi, A67410) and a kit using the colorimetric method to determine nitrate, ammonia and nitrite concentrations. The kits were obtained from AquaMerck (Merck KGaA, Darmstadt, Germany). The water temperature was maintained at 21.5 ± 2.4°C; the level of dissolved oxygen was 6.6 ± 1.3 mg/L. Water salinity was 31.5 ± 4.1 g/L, pH 7.3 ± 0.4, NO_{3-}25-150 mg/L, NO_{2-} 0.05-0.5 mg/L and the ammonium value was undetectable. All values were measured three times a week. The photoperiod was natural throughout the experimental period (16L/8D in summer and 12L/12D in winter) and all tanks had similar lighting conditions.

A group of 90 fish (average weigh 530g), were distributed in the nine experimental tanks (ten fish per tank).

2.2. Experimental diets and feeding regime.

Five fish at the beginning and all fish at the end of the experiment were slaughtered by a thermoshock in a melting ice bath, to determine body composition and biometric parameters and were stored at -30 ºC to determine proximate composition, fatty acids, heavy metals and sensory analysis.

Three isolipidic (140 g/kg of crude lipid) and isoenergetic diets (24 MJ/kg of gross energy) were formulated (Table 6.1) with the same digestible protein level (50% DP), and from 530 to 604 g/kg of crude protein (CP). For protein digestibility estimation, the individual ingredients digestibility coefficients were taken from a previous study (Tomás-Vidal et al., 2019).

The diets were formulated based on proximate analysis of different protein sources (corn gluten meal, krill meal and meat and bone meal). The FM100 diet served as a control diet containing FM as the main protein source (525g/kg), while 33% and 66% of the FM in the FM66 and FM33 diet respectively, were substituted by the alternative protein mixture (corn gluten meal, krill meal and meat meal).The FM33 diet was also supplemented with synthetic L-Met and L-Lys in amounts of 3 g/kg, to simulate the digestible amino-acid profile of the FM diet. These were the three diets with best growth and survival results in low weight yellowtails (Monge-Ortiz et al., 2018a).

The primary lipid source in all feeds was fish oil, with levels of about 90 g/kg of dry matter. The composition of the experimental diets and their proximate values are shown in Table 6.1.

The different ingredients of the diets were weighed individually and mixed to form homogeneous dough and were prepared by cooking-extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). Processing conditions were as follows: 100 rpm speed screw, 110 ºC temperature, 30-40 atm pressure and 5 and 6 mm diameter pellets, according to fish size.

Fish were fed throughout the 84-day period by hand, twice a day (9.00h and 17.00h), six days per week until apparent satiation; the

experiment finished when fish reach the commercial weight. Any uneaten feed was collected daily to determine fish feed intake (FI).

Table 6.1 Ingredients, chemical and heavy metals composition of the experimental diets.

	FM100	**FM66**	**FM33**
Ingredients (g/kg DM)			
Fish meal [1]	525	350	175
Wheat [2]	235	108	43
Wheat gluten [3]	130	130	140
Corn gluten [4]		100	100
Extracted krill meal [5]		120	230
Meat and bone meal [6]		80	198
Fish oil	90	92	88
L- Methionine [7]			3
L-Lysine Clh [7]			3
Vitamin and mineral mix [8]	20	20	20
Chemical composition (% DM)			
Dry matter (DM)	888	888	895
Crude protein (CP)	530	580	604
Crude lipid (CL)	139	142	138
Ash	103	106	121
CHO [9]	228	171	137
GE (MJ/Kg)	23.8	24.1	23.7
DP (g/Kg) [10]	497	504	497
DE (MJ/Kg) [10]	20.3	19.2	18.1
DP/DE ratio (g/MJ)	24.5	26.2	27.5
Heavy Metals			
Arsenic	3.15	2.09	1.86
Cadmium	0.60	0.41	0.44
Cooper	5.8	24.5	63.2
Mercury	0.03	0.04	0.04
Zinc	432.8	65.7	60.4

[1] Fish meal (93.2% DM, 70.7% CP, 8.9% CL, 15.1% Ash)
[2] Wheat meal (92.4% DM, 17.1% CP, 2.4% CL, 78.3% CHO, 2.4% Ash)
[3] Wheat gluten (93.3% DM, 8.1% CP, 9% CL, 73.9% CHO, 9% Ash)
[4] Corn gluten (93.3% DM, 72.9 CP, 0.9% CL, 25.3% CHO, 0.9% Ash)
[5] Extracted krill meal: product obtained by removing the fat with ethanol (87.8% DM. 69.7% CP. 2.9% CL. 8.17% CHO. 11.6% Ash); VALGRA S.A. Beniparrell. Valencia. Spain

[6] Meat and bone meal (97.0% DM. 53.1% CP. 15.3% CL. 4.7% CHO. 26.9% Ash. 17.69 kJ Energy); VALGRA S.A. Beniparrell. Valencia. Spain

[7] L-Methionine and L-Lysine Clh: Guinama S.L.U.

[8] Vitamin and mineral mix (g/kg): Premix: 25; Choline. 10; DL-a-tocopherol. 5; ascorbic acid. 5; $(PO_4)_2Ca_3$. 5. Premix composition: retinol acetate. 1000000 IU/kg; calcipherol. 500 IU/kg; DL-atocopherol. 10; menadione sodium bisulphite. 0.8; thiamine hydrochloride. 2.3; riboflavin. 2.3; pyridoxine hydrochloride. 15; cyanocobalamine. 25; nicotinamide. 15; pantothenic acid. 6; folic acid. 0.65; biotin. 0.07; ascorbic acid. 75; inositol. 15; betaine. 100; polypeptides 12.

[9] Carbohydrates (CHO) (%) = 100-%CP-%CL-%CF-%Ash

[10] Digestible protein (DP) and digestible energy (DE) were calculated based on the respective values of apparent digestibility coefficients (ADC) estimated by a digestibility trial in the previous experiment (Monge-Ortiz, 2017a: ADC protein (%) (diet 100 = 94; diet 66 = 87; diet 33 = 82) and ADC energy (%) (diet 100 = 85.5; diet 66 = 79.5; diet 33 76.5).

All fish were individually weighed at intervals of 30 days approximately. Prior to weighing, the fish were anaesthetized with 30 mg/L clove oil (Guinama®, Valencia, Spain) containing 87% eugenol. At the end of the growth trial, all fish were individually weighed. The fish were not fed for 24 hours before weighing and were slaughtered by a thermoshock in a melting ice bath to avoid affecting fillets quality.

2.3. Proximate composition and fatty acid analysis

Five fish per tank were randomly sampled to determinate the biometric parameters and to carry out the proximate composition analysis.

Diets and their ingredients, as well as the whole and fillet fish, were analyzed according to AOAC (1995) procedures: dry matter (110 ºC to constant weight), ash (incinerated at 550 ºC to constant weight), ether extract was determined using an Ankom XT10 Extraction System (NY, USA) according to AOCS (2005); nitrogen content (crude protein x 6.25) was determined using a Leco CN628 Elemental Analyzer (Leco Corporation, St. Joseph, MI, USA) according to AOAC (2005). Energy was calculated according to Brouwer (1965), from the C (g) and N (g) balance (GE = 51.8 × C − 19.4 × N). Carbon and nitrogen were analyzed by the Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA).

Fatty acid methyl esters (FAME) of total lipids were prepared directly as previously described by O´Fallon (2007). FAME were analysed in a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector. The separation of methyl esters was performed in a fused silica capillary column SPTM 2560 (Supelco, PA, USA) (100 m x 0.25 mm x 0.2 µm film thickness). The carrier gas was Helium at a linear velocity of 20 cm/seg. The samples were injected with a split ratio of 1/100. The initial oven temperature was set at 140 ºC held for five min and increased to 240 at 4 ºC/min and finally maintained at that temperature for 30 min. Both detector and injector temperatures were set at 260 ºC. The individual fatty acids were identified by comparing their retention times with standards of fatty acid methyl esters supplied by Supelco. Only those fatty acids which were present at minimum levels of 0.1% were considered. In order to quantify the fatty acids, we used the sample weight data from the analysis to calculate the g of fatty acids per 100 g of sample and quantified by using C13:0 as internal standard. Fatty acid composition of the experimental diets is shown in Table 6.2.

Table 6.2 Fatty acid composition of the experimental diets (g/kg DM).

Fatty acids	FM100	FM66	FM33
SFA			
C13:0	3.57	3.32	4.04
C14:0	7.34	7.22	6.78
C15:0	0.48	0.45	0.41
C16:0	24.2	24.7	26.3
C17:0	0.44	0.42	0.43
C18:0	4.44	5.20	6.71
C20:0	0.21	0.38	0.28
C22:0	0.20	0.22	0.14
MUFA			
C16:1	8.50	7.87	7.00
C17:1	0.13	0.14	0.17
C18:1 n7	3.60	3.71	3.92
C18:1 n9	13.14	16.29	20.87
C20:1	0.71	0.65	0.64
C24:1	0.42	0.31	0.26
PUFA			
C18:2 n6c (LA)	8.41	9.85	11.21
C18:3 n6	0.23	0.21	0.22
C18:3 n3 (LNA)	1.21	1.39	1.66
C20:2	2.48	2.83	2.90
C20:3 n6	0.17	0.15	0.13
C20:4 n6 (ARA)	1.01	0.87	0.74
C20:5 n3 (EPA)	19.65	17.74	15.07
C22:2	0.74	0.67	0.59
C22:5 n3	2.07	1.70	1.31
C22:6 n3 (DHA)	15.33	12.85	10.52
n-3 HUFA			
n3	38.49	33.89	28.78
n6	9.82	11.08	12.3
n3/n6	3.92	3.06	2.34
EPA/DHA	1.28	1.38	1.43

SFA: Saturated fatty acids; MUFA: Mono unsaturated fatty acids; PUFA: Poly unsaturated fatty acids; n-3 HUFA: Highly unsaturated fatty acids; LA: Linoleic acid; LNA: Linolenic acid; ARA: Arachidonic acid.

2.4. Heavy metals

Five heavy metals (arsenic, cadmium, cooper, mercury and zinc) were analysed in experimental diets and fish muscle. Heavy metals composition of experimental diets is shown in Table 6.1.

Six samples of fillets of each treatment were lyophilized for heavy metals analysis. 0.5 g of lyophilized sample was digested with 5 ml HNO3Suprapur® in Teflon vials to decompose the samples at 100°C for 3 hours. A flame atomic absorption spectrophotometer using a Varian Spectraa 220, instrument was used for Cu, Zn and Cd determinations according to Niencheski (2006).

For determining total arsenic, the samples were digested with nitric acid and hydrogen peroxide in a microwave digester (Start D, Milestone, Italy) at 170°C. The quantification was performed with an inductively coupled plasma mass emission spectrometry (ICP MS) (7700x, Agilent Technologies, Japan) using a collision call with He, according to AOAC (2012).

The determination of total mercury was performed using a thermal decomposition and amalgamation atomic absorption spectrometry (DMA-80, Milestone, Italy) measuring the absorption of Hg in 253.7 nm, according to Paiva et al. (2016).

Accuracy of analytical results was checked by analysing reference materials. DORM-4 –Fish protein Certified Reference Material for Trace Metals (NRC-CNRC) was used for cadmium, cooper, zinc and arsenic. In the case of mercury, TORT-2 Lobster Hepatopancreas Reference Material for Trace Metals (NRC-CNRC) was used as reference material. Results obtained for the reference samples were in good agreement (+ 8 %) with the certified values.

2.5. Fish fillet characterization

15 fish for each treatment were kept in ice during transport to the laboratory. Heads and guts were removed before freezing at -20 ºC. Prior to sampling, fish were thawed in a refrigerator at 4 ºC overnight. Then, they were hand-filleted, the skin discarded. To obtain the samples, two lateral fillets were removed and cut into two pieces.

The physicochemical properties analysed in raw samples were colour, pH, texture and moisture. Organoleptic analyses were performed in raw and cooked fish. 30samples for each treatment (FM33, FM66 and FM100 or control) were analysed.

2.5.1. Physicochemical analyses

Colour coordinates CIE L*a*b* (CIE, 1976) of 15 fish samples of each treatment were determined using a Minolta CM 700D colorimeter D (Minolta Camera Co, Osaka, Japan). Illuminant and observer were D-65 and 10º (UNE 7203). CIE Lab coordinates L* (lightness), a* (redness), and b* (yellowness) were used to calculate colour difference, ΔE (compared to control sample, FM100) (Equation 1) and chroma (Equation 2). Six measures were performed at different points of each sample.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^{*2} + \Delta b^{*2}} \quad \text{(Equation 1)}$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad \text{(Equation 2)}$$

The moisture content was determined by oven drying at 105 ºC for 20-24 h (AOAC, 2012). Determinations were obtained by duplicate.

The pH of samples was measured with a pHmeter micropH 2001 (Crison Instruments, S.A., Barcelona, Spain) with a puncture electrode (Crison 5231) at five different points of the sample.

2.5.2. Mechanical properties

Texture analysis was carried out using a TA-XT Plus Texture Analyser (Stable Micro Systems Ldt., Godalming, UK) with a 50 kg load cell. Samples were compressed to 60% of initial height with a cylindrical aluminium probe (P/6) of 6 mm diameter. The test conditions involved two consecutive cycles of 30% compression with a 5 seconds interval between cycles. The pre-test speed, test speed and post-test speed were 1, 1 and 5 mm/s, respectively. Force vs distance curves were recorded with Texture Exponent Lite 32 version 4.0.8.0. The analyses were performed in fish pieces of 50x50x15 mm (wide x long x thick). Seven textural parameters were determined from each curve: hardness (N), adhesiveness (N.s), springiness, cohesiveness, gumminess, chewiness and resilience (Bourne, 1978). 15 samples were analysed for each treatment.

2.5.3. Organoleptic analyses

The effect of diet on sensory properties of fish fillets was studied comparing FM100 (control diet), with FM66 and FM100 with FM33 samples. The products, raw and cooked samples, were subjected to sensorial evaluation through a test of degree of acceptability and using a hedonic scale of 5 points. The test was performed by 11 selected and trained panel members.

The sensory attributes analysed on raw samples were: marine aroma (1: not present and 5: very intense), strange odour or degradation odour (1: not present and 5: very intense), brightness and whiteness (1: very intense and 5: subdued colour), compactness (1: compact and 5: crumbled), water retention (1: wet and 5: dry), superficial sliminess (1: not present and 5: evident), muscle integrity (1: without separation and 5: with evident separation) and elasticity (1: very elastic and 5: rigid).

Cooked samples were heated prior sensory evaluation in a microwave oven until reaching an internal temperature of 65 – 70 ºC (CAC-GL 31-1999). The sensory attributes analyzed were: lightness (1: translucent

and 5: dull), whiteness (1: very intense and 5: subdued color), marine flavor (1: not present and 5: very intense), unpleasant or strange taste (1: not present and 5: intense), compactness (1: compact and 5: crumbled), water retention (1: wet and 5: dry), greasiness (1: very greasy and 5: little greasy) and adhesiveness (1: not adherent and 5: very adherent).

2.6. Statistical analysis

Growth data, feed utilization and all the data obtained were evaluated using one-way analysis of variance (ANOVA), with the initial live weight as covariate (Snedecor & Cochran, 1971). The Newman–Keuls test was used to assess specific differences among diets at a level of $p < 0.05$ (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA).

2.7. Ethical statement

This study has complied with the European Union Council Directive 2010/63/ UE, which lays down minimum standards for animal protection under experimentation, and it was also in accordance with Spanish national legislation (Spanish Royal Decree 53/2013), which regulates animal usage in experimentation and/or for other scientific purposes. Fish in the tanks were checked on a daily basis. Every four weeks, fish were weighed individually and their health status was assessed by observation, after sedation with clove oil dissolved in water (1 mg / 100 ml of water) to minimize animal suffering. Animals were killed by a thermal shock in ice and then dissected.

3. RESULTS

3.1. Fish growth

Growth performance of Mediterranean yellowtail fed experimental diets is shown in Table 6.3. Significant differences ($p<0.05$) were found

between FM33 diet and the other two diets in final weight and specific growth rate (SGR), obtaining a worst growth in the high substitution diet. Survival was 100% in fish fed FM100 and FM66 diets, and 90% in fish fed FM33. Mortality took place after fish sampling, probably due to the fish handling. Also, protein efficiency ratio (PER) was statistically different between fish fed the FM33 diet with greatest substitution (0.9) and the other two diets (1.3).

Table 6.3 Overvall performance of *Seriola dumerili* fed the experimental diets.

	FM100	**FM66**	**FM33**	**SEM**
Initial body weight (g)[1]	525.4	517.1	547.1	19.8
Final body weight (g)	853.1[a]	854.8[a]	750.8[b]	15.6
SGR (%/day)[2]	0.56[a]	0.56[a]	0.41[b]	0.02
FI (g/100g fish day)[3]	0.85	0.80	0.85	0.07
FCR[4]	1.55	1.45	1.65	0.21
PER[5]	1.3[a]	1.3[a]	0.9[b]	0.05

[1]Means with different superscripts are significantly different ($p < 0.05$). SEM: standard error of the mean. Newman-Keuls test. n=3 1Initial weight was considered as covariable for final weight and specific growth rate.
[2]Specific growth rate (SGR) =100 x ln (final weight/initial weight) /days.
[3]Feed intake (FI) (g 100 g fish-1 day-1) = 100 x feed consumption (g)/average biomass (g) x days.
[4]Feed conversion ratio (FCR) = feed consumption (g) / weight gain (g).
[5]Protein efficiency ratio (PER) = weight gain (g) / crude protein intake (g).

3.2. Biometrics, body composition and nutrient retentions.

Data on biometric parameters and body composition of fish fed different diets are shown in Table 6.4. No significant differences were observed on the body composition and most biometric parameters ($p<0.05$). Significant differences were solely found in condition factor (CF), fish fed the FM100 and FM66 diets were more than 1.50 g cm^{-3}, significantly higher than CF of fish fed the FM33 diet (1.38 g cm^{-3}).

Table 6. 4 also shows the retention efficiency of protein, lipid and energy, where statistical differences were observed in lipid retention efficiency, they were significantly higher in fish fed the FM66, and the lowest value appeared in fish fed the FM33 diet. As regards energy

retention efficiency, differences appear between the control diet (21%) and the other two diets (17.2% FM66 and 16.5% FM33).

Table 6.4 Whole-body composition (% wet weight) and biometric of *Seriola dumerili* fed the experimental diets.

	FM100	FM66	FM33	SEM
Whole-body composition (n=3)				
Moisture (%)	71.3	71.0	74.4	1.4
Crude protein (%)	19.2	19.5	17.3	0.7
Crude lipid (%)	6.8	6.4	4.3	0.6
Ash (%)	2.8	2.9	3.3	0.3
Energy (kJ/g)	6.3	6.3	6.0	0.2
Biometric indices (n=15)				
CF (g/cm^3)[2]	1.55^a	1.58^a	1.38^b	0.06
VSI (%)[3]	4.84	5.41	4.83	0.42
HSI (%)[4]	0.98	0.92	1.01	0.05
VFI (%)[5]	0.09	0.13	0.02	0.05
MI[6]	46.6	46.6	47.2	1.1
PNEP[7]	50.3	49.6	50.5	0.8
DP (%)[8]	65.2	64.9	64.5	1.3
Nitrogen, lipid and energy retention, % intake (n=3)				
Crude protein efficiency[9]	31.7	32.5	23.8	3.0
Crude lipid efficiency[10]	36.4^b	53.3^a	7.1^c	7.8
Gross energy efficiency[11]	21.0^a	17.2^b	16.5^b	2.8

[1] Means in the same row with different superscript letters are significantly different ($p<0.05$). SEM: standard error of the mean. Newman-Keuls test.
[2] Condition factor (CF) (g cm3 -1) = (total fish weight (g) / length3 (cm)) x 100
[3] Viscerosomatic index (VSI) (%) = (visceral weight (g) / total fish weight, (g)) x 100
[4] Hepatosomatic index (HIS) (%) = (liver weight (g) / total fish weight (g)) x 100
[5] Visceral fat index (VFI) (%) = (visceral fat (g) / total fish weight (g)) x 100
6Meat index, MI = (fillet weight (g) / total fish weight (g)) x 100
[7] Percentage of non-edible portion (PNEP) = ((head+ fins+ rachis+ visceral weight, g) / total fish weight (g) x 100.
[8] Dressout percentage (DP) (%) = ((total fish weight- head-viscera weight (g) / total fish weight (g)) x 100.
[9] Crude Protein Efficiency (%) = (fish protein gain (g) x 100) / protein intake (g).
[10] Crude lipid Efficiency (%) v = (fish lipid gain (g) x 100) / lipid intake (g).
[11] Gross Energy Efficiency (%) = (fish energy gain (kJ) x 100) / energy intake (kJ).

3.3. Fatty acid composition

Saturated (SFA), mono-unsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in muscle of Mediterranean yellowtail showed a very significant ($p<0.05$) decrease in most of the fatty acids between fish fed FM33 diet and the other two experimental diets, with the decrease of FM level (Table 6.5).

The total concentration of n3 PUFA in muscle showed significant differences between FM33 and the other two treatments. ARA and DHA showed differences between theFM66 and FM33 treatments, and the highest level was shown in FM66.These lower values in FM33 fillets must be due to the low fat level of the fish (4.3%) although no differences appeared between fish fed the different diets, the dismissing tendency was very clear.

The n3/n6 ratio was higher in fish fed the FM66 diet with respect to the FM100 diet ($p<0.05$). EPA/DHA ratio showed similar values between the FM66 and FM33diet; moreover, these ratios were significantly higher in the FM100 diet.

Table 6.5 Fatty acid composition (mg 100/g of dry matter) of yellowtail fillets fed the experimental diets.

	FM100	FM66	FM33	SEM
SFA[1]	6.23^{a}	6.56^{a}	3.78^{b}	0.36
C:16:0	3.84^{a}	3.95^{a}	2.23^{b}	0.29
C18:0	1.30^{ab}	1.57^{a}	0.95^{b}	0.07
MUFA[2]	7.51^{a}	6.86^{a}	3.54^{b}	0.48
C16:1	1.13^{a}	0.10^{b}	0.46^{ab}	0.06
C18:1 n7	0.80^{a}	0.91^{a}	0.36^{b}	0.14
C18:1 n9c	4.98^{a}	5.20^{a}	2.48^{b}	0.44
C20:1	0.31^{a}	0.36^{a}	0.10^{b}	0.04
PUFA[3]	9.28^{a}	9.06^{a}	4.38^{b}	0.47
C18:2 n6c (LA)[4]	3.22^{a}	2.77^{a}	1.44^{b}	0.28
C18:3 n3 (LNA)[5]	0.40^{a}	0.31^{ab}	0.17^{b}	0.11
C20:4 n6 (ARA)[6]	0.19^{ab}	0.23^{a}	0.12^{b}	0.02
C20:5 n3 (EPA)[7]	1.40^{a}	1.04^{a}	0.51^{b}	0.12
C22:5 n3	0.53^{a}	0.54^{a}	0.28^{b}	0.03
C20:2	0.24^{a}	0.22^{a}	0.07^{b}	0.02
C22:2	0.12^{a}	0.11^{a}	0.04^{b}	0.01
C22:6 n3 (DHA)[8]	3.01^{ab}	3.71^{a}	1.69^{b}	0.14
n3 HUFA[9]	4.94^{a}	5.29^{a}	2.48^{b}	0.37
n3	5.38^{a}	5.64^{a}	2.67^{b}	0.35
n6	3.54^{a}	3.11^{a}	1.61^{b}	0.35
n3/n6	1.53^{b}	1.85^{a}	1.65^{ab}	0.09
EPA/DHA	0.46^{a}	0.28^{b}	0.30^{b}	0.03

Means in the same row with different superscript letters are significantly different ($p<0.05$). Newman Keuls test. n=3.

[1]SFA: Saturated fatty acids; [2]MUFA: Mono unsaturated fatty acids; [3]PUFA: Poly unsaturated fatty acids; 9n-3 HUFA: Highly unsaturated fatty acids; [4]LA: Linoleic acid; [5]LNA: Linolenic acid; [6]ARA: Arachidonic acid; [7]EPA: Eicosapentaenoic acid; [8]DHA: Docosahexaenoic acid. Crude lipid muscle (% of dry matter): 25.5% FM100; 22.6% FM66 and 12.2% FM33.

3.4. Heavy metals

Copper increased its values in the different diets with a wide difference between them (Table 6.1), in FM66 values appearing 4 times higher than FM100, and in FM33 values appearing 10 times higher than FM100 diet ($p<0.05$). Unlike in zinc values where FM100 showed the highest value, 7 times higher than FM33and FM66 diet. Arsenic

showed clearly lower values in the diets with a FM replacement higher (FM33 and FM66). Arsenic, cadmium and mercury showed similar values amongst all diets.

Table 6.6 shows the mean concentrations of the five heavy metals analyzed (arsenic, cadmium, copper, mercury and zinc) in the Mediterranean yellowtail fillets fed the experimental diets. Relatively low levels of arsenic and copper were observed in the tissues of fish samples while insignificant concentrations (<0.2 mg/kg DM) of mercury and cadmium were obtained. The concentration of zinc in muscle varied from 25.2 to 30.5 mg/kg DM, without differences between diets ($p<0.05$).

Table 6.6 Heavy metals composition of mediterranenan yellowtail (mg/1000g DM) fed the experimental diets.

	FM100	FM66	FM33	SEM
Arsenic	1.40[a]	1.38[a]	0.81[b]	0.08
Cadmiun	0.04	0.00	0.01	0.01
Cooper	1.34	1.42	2.60	0.35
Mercury	0.13	0.11	0.12	0.01
Zinc	25.2	26.6	30.5	1.7

Means of the same row with different letters are significally different ($p<0.05$). SEM: pooled estandar error of the mean. Newman-Keuls test. n=6.

3.5. Sensory analysis: Physicochemical and mechanical analysis.

Table 6.7 shows the pH, color and texture measurements in *S. dumerili* fillets. In pH, a difference between FM33 and experimental diets can be observed, where the FM33 value was significantly lower. With respect to color measurements, significant differences ($p<0.05$) between the high substitution diet (FM33) and the other two diets in yellowness and chromaticity were found, the FM33 value was significantly higher in yellowness and lower in chromaticity; also differences between control diet and experimental diets in lightness and redness were found, where FM100 value was significantly higher in both cases. No differences were observed in Delta E ($p<0.05$).

In texture measurements (Table 6.7), significant differences between FM33 and the other experimental diets ($p<0.05$) were found in adhesiveness, chewiness, gumminess and hardness, yellowtail fillets feed with the high substitution diet (FM33) being significantly lower in adhesiveness and higher in the other three parameters. No significant differences were shown in cohesiveness, springiness and resilience ($p<0.05$).

Table 6.7 Chemical, physical and textural characteristic from *S. dumerili* fillets at the end of the experiment.

	FM100	FM66	FM33	SEM
pH and Color				
pH	6.4^{b}	6.4^{b}	6.6^{a}	0.030
Lightness (L)	40.1^{a}	38.0^{b}	37.4^{b}	0.481
Redness (a)	-4.2^{b}	-3.5^{b}	-3.3^{a}	0.112
Yellowness (b)	3.6^{b}	4.1^{b}	5.67^{a}	0.290
Delta E	3.92	3.8	2.9	0.282
Chormaticity (C)	16^{b}	17.5^{b}	22.9^{a}	1.386
Texture				
Adhesiveness	-1.32^{a}	-1.27^{a}	-2.46^{b}	0.309
Chewiness	64.15^{b}	70.88^{b}	88.85^{a}	5.219
Cohesiveness	0.636	0.64	0.6	0.011
Gumminess	87.48^{b}	94.49^{b}	115.14^{a}	6.573
Hardness	138.9^{b}	152^{b}	191.5^{a}	11.728
Springiness	0.73	0.75	0.77	0.013
Resilience	0.29	0.29	0.28	0.005

Means in the same row with different superscript letters are significantly different ($p<0.05$). SEM: pooled standard error of the mean. Newman-Keuls test. n= 15.

3.6. Sensory analysis: organoleptic evaluation.

In the sensory analysis (Fig. 6.1) of raw samples, the marine aroma was higher in the experimental diets as compared to the FM100 diet, while whiteness and water retention, higher in the FM100 diet than in FM66 diet.

In cooked samples, significant differences ($p<0.05$) were found in whiteness between FM100 and FM66 and in lightness between FM100 and FM33, being FM100 the highest value in both cases.

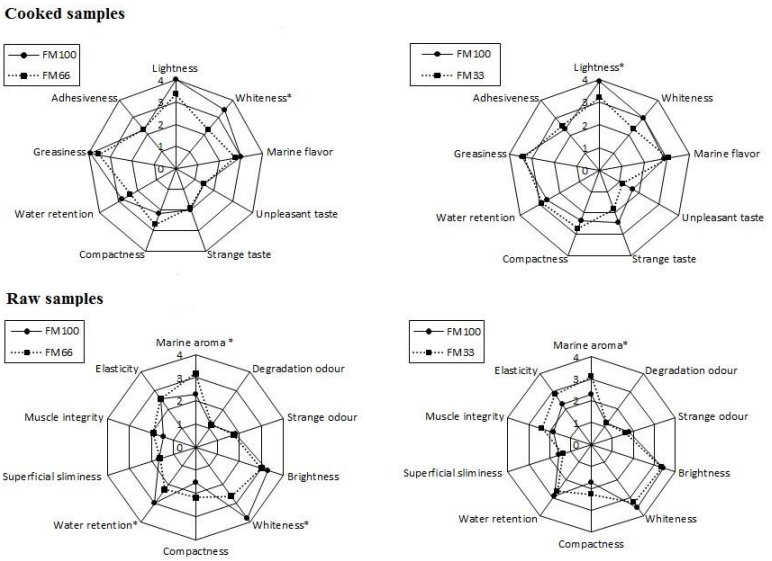

Figure 6.1 Organoleptic test of *S. dumerili* samples of muscle. Comparation between FM100-FM66 and FM100-FM33 in raw and cooked samples. Significant differences ($P < 0.05$) are shown with * in the graphics. Newman-Keuls test.

4. DISCUSSION

Fish performance

Fish growth in the present trial was similar or superior to other studies on similar size Mediterranean yellowtail (Jover et al., 1999, Tomás et al., 2005, 2008), but inferior to that of previous studies with the same diets (Monge-Ortiz et al., 2018a), because the fish size differences. The good growth with the FM66 diet was also in accordance with previous studies using vegetal or animal proteins of up to 30% obtaining no differences in *S. dumerili* (Dawood et al., 2015; Tomás el al., 2005) or S. quinqueradiata (Aoki et al., 2000; Shimeno et al., 1993; Viyakarn et al., 1992; Watanabe et al., 2000).

However, yellowtail fed a diet with the lowest FM level had the worst growth.

S. dumerili is a basically a piscivorous predator (Andarolo & Pipitone, 1997). Adults and juveniles feeding habit consists mainly on Teleostei, but also Cephalopoda and Crustacea (Matallanas et al., 1995) classified in the highest trophic value (Stergiou & Karpouzi, 2002). As a carnivorous fish, high dietary levels of FM replacement by plant and terrestrial alternative proteins is considered as a stress factor leading poor fish growth and survival (Estruch et al., 2018a). It is well-know the physiological consequences of plant-based diets or other alternative feed ingredients, such us, impact on immune system (Sitja-Bobadilla et al., 2005) alterations of the gut microbiota (Estruch et al., 2015) , gut gene expression (Estruch et al., 2018b) and gut proteomic profile (Estruch et al., 2020), intestinal histology alteration (Baeza et al., 2016), intestinal colic or gastric dilatation (Baeverfjord et al., 2006), suggesting detrimental effects on intestinal function that increase in the long term feeding (Estruch et al., 2020).

In a previous study with the same diets and smaller fish, yellowtail fed diet FM33 showed lower growth than fish fed FM66 and FM100, although significant differences were not observed (final weight 345g above 391 and 385g) (Monge-Ortiz et al., 2018a). Probably, long term feeding period with FM33 diet (154 days for the first experiment plus 84 days for the present experiment), because it is known that vegetal or other animal ingredients may increase the risk of various digestive disorders including intestinal colic in Atlantic cod and gastric dilatation in rainbow trout (Baeverfjord et al., 2006), most of which have been related to feed (antinutritional factors in vegetal sources and worst digestibility in krill meal for example). The disorders are often not lethal but may increase the susceptibility to secondary disorders and lower growth.

No differences appear in feed intake (FI) suggesting a good acceptance of a partial FM substitution diet in yellowtail (Monge-Ortiz et al., 2018a). Likewise (FCR) was similar in all diets. However, the worst results observed in PER with the highest FM substitution diet support the poor protein utilization of FM33, as it has been previously reported, due to the lack of one or more EAAs (Martínez-Llorens et al.

2012), of poor metabolic adaptation of liver to higher plant proteins (Panserat et al., 2009), and digestibility and bio-availability of alternative protein (Tomás et al, 2019)

The risk of cited digestive disorders might palliate including dietary additives tested successfully in carnivorous fish, like sodium butyrate that restores the intestinal homeostasis and disease resilience, (Piazzon et al., 2017), galactomannanoligosaccharides to attenuate stress response (Serradell et al., 2020) and to increase feed digestibility (Burr et al., 2008), proteins in acid silage that improve performance (Olsen & Tope, 2017), or including low amount alternative marine ingredients to recover the gut mucosa functionality (Estruch et al., 2020).

Biometric indexes

The hepatosomatic index (HSI), viscerosomatic index (VSI) and visceral fat index (VFI) did not present differences between treatments, showing that different substitution levels did not affect the liver, viscera or fat weight. Also, no differences have been obtained in previous similar seriola studies (Aoki et al., 2000; Dawood et al., 2015; Tomás et al., 2005) where FM has been substituted by plant meals. Differences in CF in the present study have been due to the lower size of the FM33 fish and worse efficiency, which has resulted in thinner fish.

VFI results were low (between 0.02 and 0.13%), indicating the low fat content of this species, as compared with sea bream (2.6%) or seabass (5.5%). Although the absence of differences between treatments, visceral fat in yellowtail feed with FM33 diets were more than 4 times lower than the other treatment, that concords with the result of the significantly lower condition factor and the very significant reduction in crude lipid efficiency retention in fish fed the FM33 diet.

Body and fillet composition

With respect to body composition, crude lipid composition does not show significant differences but a decrease when the fish meal level decreased in the diets in the FM33 diet can be appreciated; this decrease maybe due to the lower growth.

The fatty acid composition in muscle of *S. dumerili* was directly related with the fatty acid profile in the different diets, as seen previously in *S. dumerili* (Monge-Ortiz et al., 2018b) and in another species like *Salmo salar* (Bell et al., 2001; Nanton et al, 2007), *Sparus aurata* (Benedito-Palos et al., 2008; Fountoulaki et al., 2009) or *Dicentrarchus labrax* (Mourente & Bell, 2006). It is common to see changes in fatty acids profiles with the fish oil substitution by other lipid sources, but there is little information about these effects of changes in fatty acids profiles affected by FM substitution.

The high quantity of n6 fatty acids in the plant sources, could unbalance the n3/n6 proportion on muscle (Robaina et al., 1998), although in this case it is slightly offset with the mix of the animal meals with plant meals, which would partially compensate this deficit.

In the present study, it can be appreciated how the decrease of FM inclusion, produces a significant decrease in most of the fatty acids, with special attention to n-3 PUFA (EPA and DHA), provided by fish oil and FM, that were significantly lower in the muscle of fish fed with the FM33 diet. This generalized reduction may be due to the lower fat content of the FM33 fish as well as the lipid profile of this diet, that cause a significant reduction in fatty acids.

The reduced levels of n3 HUFA, especially EPA and DHA that are only present in fish oils, with the consequent decrease in the n3/n6 ratio, has been observed in previous studies with other species, like rainbow trout, sea bream, (De Francesco et al. 2004, 2007) or Senegalese sole (Valente et al., 2011). Such alterations, found in all fish species evaluated (Turchini et al., 2007), either as salmon and other freshwater species may compromise the nutritional quality of fish for

human consumption, that has a great importance, especially EPA and DHA, in the prevention of several diseases.

As regards heavy metals, the EU has established maximum levels in commercial foodstuffs for several of the heavy metals discussed in this paper. In the present study, the levels of heavy metals do not exceed in any case those allowed by the EU, and there are no differences between diets in heavy metal concentrations in fillets, except in arsenic, whose concentration was lower in fish fed the FM33 diet than in fish fed the FM66 and the control (FM100) diets. Arsenic is bioaccumulated by marine organisms (Edmond & Francesconi, 2003) and the yellowtails fed a diet with lower FM had lower arsenic concentrations. Levels of total arsenic between 3 and 8 mg/kg are typical for FM in the literature, however the species of toxicological relevance, i.e. inorganic arsenic, usually constitutes less than 1.2% of the total arsenic concentration (Sloth et al. 2005).

Present levels of cadmium, mercury and arsenic are far below the acceptable levels; so that, the metals present in the fillets of the different diets, do not represent a health hazard.

In other studies, it has been observed that high levels of metals in diets, like zinc in the FM100 or copper in the FM33 diet in this case, do not significantly affect fish composition, as they are not bioaccumulated in muscle. Muscle has the lowest bioaccumulation level of metals, the main target organs being the liver and the gills (El-Moselhy et al. 2014; Yousafzai et al., 2012). Copper and zinc are accumulated mainly in the liver, likely linked to its role in metabolism, related to natural binding proteins such as metallothioneins and act as a reservoir for these metals to fulfill their demand in metabolic or enzymatic processes (Amiard et al., 2006; Görür et al., 2012; Roesijadi, 1996).

On the other hand, cadmium trend to accumulate in gills, because they have very large surface areas that facilitate rapid diffusion of toxic

metals, and probably, it suggests that metals accumulated in gills are mainly concentrated in water (Dural et al. 2007; El-Moselhy et al., 2014).

Sensorial traits and organoleptic test

As regards chemical analysis, pH and color results indicate that significant differences are present between diets but, they did not affect the appearance of the meat from the point of view of the consumer. All the pH values are in the normal range for fresh fish (Abbas et al., 2008) and there are not differences in the DE values. These differences may be attributed to fish variability rather than a diet effect. The water retention value does not show significant differences between treatments; values like color and texture parameters are also related with water holding capacity.

The present data show that the replacement of FM affects texture characteristics, but only in the highest FM substitution. Similar results were obtained in Japanese seabass (Hu et al., 2013) when were fed with 80% of dietary FM substitution althoughsevere changes were not observed in organoleptic properties. The exact mechanism for this is still unknown, and further investigation is needed, but these parameters may be affected by the different lipid content and fatty acid profile of experimental diets (Hu et al., 2013) since it has been observed in other seriola studies that muscle protein content does not affect meat texture (Thakur et al., 2009).

The organoleptic tests, in both, raw and cooked samples, were similar.

In raw samples, the panelists only found differences between diets in both cases in marine aroma, that is more present in experimental fillets than in FM100 fillets and may be caused by krill meal. Panelist also find differences in the FM66 fillets in whiteness and water retention, despite the fact that neither characteristic presented

differences in the chemical analysis. In previous studies with seabream, seabass or turbot, similar results had been reported with stronger smell in the fillets when fish were fed non fish-meal diets (Izquierdo et al., 2003; Regost et al., 2003).

In cooked samples, panelists were only able to find differences between both treatments in parameters related with color but not with flavor, finding differences in whiteness between FM66-FM100 and in lightness between FM100-FM33, this being consistent with color parameters tested in raw samples.

In other similar studies, no differences in taste or texture have been reported by the panelists in seabream or seabass with fish oil substitution by vegetal oils (Izquierdo et al., 2003), or in Atlantic halibut (Martins et al., 2011) or turbot (Regost et al., 2003).

This absence of differentiation in flavor between diets in cooked samples, despite the differences reported in physicochemical and textural characteristics, lipid content and fatty acids profile, may be not very surprising as even big changes in the flesh fatty acids profile does not induce big changes in sensory response in panelists to cooked fillets as seen in other species like seabream (De Francesco et al. 2007; Izquierdo et al., 2003; Martins et al., 2011)

In summary, fish meal substitution is possible in yellowtail diets in long periods without affecting growth and quality parameters, only when these substitutions are too high it is possible to have serious problems, especially in the productive parameters.

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Capítulo 7

Replacement of fish oil with vegetable oil blends in feeds for greater amberjack (*Seriola dumerili*) juveniles: Effect of growth performance, feed efficiency, tissue fatty acid composition and flesh nutritional value.

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ABSTRACT

This study was undertaken to assess the effects of fish oil (FO) substitution by a mixture of alternative vegetable oils (VO) on *Seriola dumerili* culture performance. A 154-days feeding experiment was conducted using juveniles (39.2 ± 1.6 g average weight). Three isolipidic and isoenergetic meal-based diets were formulated varying their lipid component. The control diet contained 100% FO (FO100), whereas diets VO50 and VO100 included a 50% and 100% blend of palm oil (PO) and linseed oil (LO) as substitute for FO, respectively.

Dietary regime did not significantly affect growth performance, biometric indices, feed efficiency, plasma chemistry and liver and muscle lipid contents. Nonetheless, dietary VO inclusion impacted the fatty acid profile of target tissues, especially in the liver. Fatty acid profiles of the fillets reflected those of the dietary oils except that there was apparent selective utilization of palmitic acid (C16:0) and oleic acid (C18:1n-9) and apparent selective retention of long chain polyunsaturated fatty acids, especially eicospentaenoic acid (EPA, C20:5 *n-3*) and docosahexaenoic acid (DHA, C22:6 *n*-3).

The nutritional value and the potential ability to prevent the development of coronary heart diseases of the flesh lipid fraction decreased with gradual FO substitution.

Keywords: alternative oil sources, fatty acid composition, fish oil substitution, greater amberjack, palm oil, linseed oil.

1. INTRODUCTION

Marine fish oils (FO) have conventionally been used as the major dietary lipid component in aquaculture feeds, especially for fast-growing marine carnivorous fish which require the supply of long chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA), and arachidonic acid (20:4n-6, AA), considered essential fatty acids (EFA) for most marine finfish species. Supplying EFA-balanced diets is indispensable to sustain not only growth, survival and feed efficiency but also health and flesh nutritional quality in cultured specimens (Sargent et al., 2002; Tocher, 2010).

Formulating suitable compound feeds is currently one of the main challenges for the aquaculture industry. The fast expansion of aquaculture production worldwide and the increasing demand of marine products along with the declining availability of fish meal (FM) and FO, make both economically and environmentally unsustainable to rely on finite marine natural resources (Kaushik et al., 2004; Tacon & Metian, 2008).

Consequently, replacement of marine ingredients by terrestrial sources in aquafeeds is being a fairly widespread practice looking for suitable alternatives for the long-term sustainability of the aquaculture industry and vegetable oils (VO) have received an important attention as substitutes of the marine oil due to their comparative reduced cost, lower concentration of dioxins and other organic pollutants, and their suitable production levels (Sales & Glencross, 2011). Numerous of these studies have covered a wide variety of fish species such as gilthead seabream (Benedito-Palos et al., 2007, 2008; Fountoulaki et al., 2009), European seabass (Izquierdo et al., 2003; Mourente & Bell, 2005), red sea bream (Huang et al., 2007), turbot (Regost et al., 2003), cobia (Trushenski et al., 2011) and Atlantic salmon (Ruyter et al., 2006; Torstensen et al., 2000). Little or no effect on fish performance has been observed in most of these investigations as far as the minimum EFA requirements were covered. Nontheless, fish fed VO have shown important modifications in their tissue fatty acid (FA) composition, including increased levels of C18 PUFA and

reduced proportions of n-3 LC-PUFA, especially EPA and DHA, which may affect not only fish health (Bell et al., 2001; Bell & Sargent, 2003; Martins et al., 2012) but also compromise the nutritional quality of flesh for human consumption, since n-3 LC-PUFA are human health-promoting compounds (Khankari et al., 2015; Simopoulos, 2008, 2011, 2016; Siriwardhana et al., 2012).

A blend of palm oil (PO) and linseed oil (LO) at a proportion of 4:1 was used in our present work to minimize potential changes derived from dietary substitution of FO. PO has high levels of C16 saturated fatty acids (SFA) and C18 monounsaturated fatty acids (MUFA), which are preferred substrates for energy production in fish species favoring diet-to-tissue transfer of LC-PUFA (Henderson, 1996; Kiessling & Kiessling, 1993) whereas LO is rich in PUFA, especially linolenic acid (C18:3n-3) which may result in tissues and organs of more favorable balanced FA. This combination of VOs should supply sufficient energy to maintain high growth, an n-6/n-3 PUFA ratio < 1 which is regarded as beneficial to human health and should not be detrimental for fish health (Bell et al., 2003b), and moderate levels of linoleic acid (C18:2n-6) trying to avoid an excessive deposition of this fatty acid which is reported as one of the most negative indicators to be taken into account when evaluating alternative lipid sources to FO for aquafeeds (Turchini et al., 2009).

The Carangidae family is a group of fish with exceptional consumer acceptance, considered of great potential for aquaculture diversification (updated by Sicuro & Luzzana, (2016). Recently, several species within this family have been abundantly targeted for research, including the effects of replacing marine ingredients by terrestrial sources in yellowtail kingfish (*Seriola lalandi*) (Bowyer et al., 2012a, b, 2013; Collins et al., 2014), Japanese yellowtail (*Seriola quinqueradiata*) (Khaoian et al., 2014; Nguyen et al., 2015; Sarker et al., 2012; Seno-O et al., 2008) and pompano (*Trachinotus* spp.) (Lech & Reigh, 2012; Lin et al., 2012; Rossi & Davis, 2012). A further carangid species, the greater amberjack, *Seriola dumerili*, is a carnivorous pelagic fish with a broad geographical distribution, fast growth rate and large size which makes it suitable for product diversification and development of value-added products, excellent flesh quality and high market price (Nakada,

2000). However, very scarce knowledge about EFA requirements or FO substitution in this species is available, being the studies published till date focused on the optimization of protein inclusion rates and the search of alternative plant protein sources to FM (Takakuwa et al., 2006b; Tomás-Vidal et al., 2008; Tomás et al., 2005; Uyan et al., 2009).

Therefore, the present study was conducted to determine whether partial (50%) or total dietary FO substitution by a blend of PO and LO (4:1) affects growth performance, feed efficiency, plasma chemistry and the degree of modification of the FA profile of liver and muscle of greater amberjack (*S. dumerili*) juveniles, including flesh lipid nutritional value. To the best of our knowledge, the present work may be considered as the first attempt to assess on the impact of FO replacement in this species.

2. MATERIALS AND METHODS

2.1 Fish and rearing conditions

A total of 185 *S. dumerili* juveniles were obtained from a fish farm (Futuna Blue S.A. Cádiz, Spain) and transported to the Fish Nutrition Laboratory of Universitat Politècnica de València (UPV, Spain). Prior to the feeding trial, fish were acclimatized to the experimental rearing conditions for four weeks by feeding a standard commercial diet. After this period, groups of 20 fish (average weight 39.2 ± 1.6 g) were randomly distributed into nine 1750 L cylindrical fibreglass tanks, three tanks per treatment.

The culture was carried out under natural photoperiod conditions in a re-circulating seawater system of 75 m^3 capacity equipped with a rotary mechanical filter and a gravity bio-filter (6 m^3). During the course of the trial, water temperature (21.5 ± 2.4 °C), salinity (31.5 ± 4.1 g/L), pH levels (7.5-8.0) and dissolved oxygen (6.6 ± 1.3 mg/L) were monitored daily.

2.2. Experimental diets and feeding regime

Three iso-lipidic and iso-energetic practical feeds were formulated to contain 510 g crude protein and 140 g crude lipid per kilo feed in a dry-weight basis. All ingredients were weighed individually before thoroughly mixed with water to form homogeneous dough and pelleted using a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France) at the Institute of Animal Science and Technology (UPV). All diets were stored at -20 °C for the duration of the trial. Fish were fed by hand to apparent satiation one of the three experimental diets for 154 days, twice a day (09:00 h and 17:00 h), 6 days a week. Any uneaten feed was collected daily to determine fish feed intake (FI).

Table 7.1 Ingredients, proximate and main fatty acid composition of experimental diets.

	FO100	VO50	VO100
Ingredients (g/kg)			
Fish meal	525	525	525
Wheat meal	235	235	235
Wheat gluten meal	130	130	130
Fish oil	90	45	0
Linseed oil	0	9	18
Palm oil	0	36	72
Vitamin and mineral premix[a]	20	20	20
Proximate composition			
Dry matter (DM, g/kg ww)	886	894	899
Crude protein (g/kg DM)	452	456	461
Crude lipid (g/kg DM)	123	133	135
Ash (g/kg DM)	91	87	87
Crude fibre (g/kg DM)	237	245	240
Fatty acids (% total fatty acids)			
Total SFA[b]	30.17	33.28	36.13
14:0	5.65	4.24	2.33
16:0	19.96	24.26	28.56
18:0	3.67	3.77	4.17
Total MUFA[b]	22.42	26.09	29.29
16:12[c]	7.74	5.70	3.12
18:12[c]	13.24	18.73	24.94
20:12[c]	0.95	0.79	0.49
22:12[c]	0.50	0.42	0.37
Total PUFA[b]	44.71	38.51	32.87
18:2 n-6	7.04	9.31	11.77
18:3 n-3	1.08	3.14	5.99
18:4 n-3	2.11	1.46	0.83
Total n-6 LC-PUFA	1.13	0.85	0.51
20:4 n-6	0.78	0.56	0.30
22:5 n-6	0.35	0.28	0.21
Total n-3 LC-PUFA[b]	29.19	20.77	12.24
20:5 n-3	15.05	10.70	5.85
22:5 n-3	1.88	1.37	0.84
22:6 n-3	11.06	7.87	5.11
Ratios			
PUFA/SFA	1.48	1.16	0.91
n-6/n-3	0.25	0.40	0.64
DHA/EPA[d]	0.735	0.736	0.874
EPA/ARA[d]	19.178	19.038	19.228

[a] Contains: choline, 10 g; DL-α-tocopherol, 5 g; ascorbic acid, 5 g; Ca3(PO4)2, 5 g and a premix, 25 g. This premix contains per kg: retinol acetate, 1,000,000 IU; calcipherol, 500 IU; DL-α-tocopherol, 10 g; menadione sodium bisulphite, 0.8 g; thiamine

hydrochloride, 2.3 g; riboflavin, 2.3 g; pyridoxine hydrochloride, 15 g; cyanocobalamine, 25 mg; nicotinamide, 15 g; pantothenic acid, 6 g; folic acid, 650 mg; biotin, 70 mg; ascorbic acid, 75 g; inositol, 15 g; betaine, 100 g; polypeptides 12 g; Zn, 5 g; Se, 20 mg; I, 500 mg; Fe, 200 mg; CuO, 15 g; Mg, 5.75 g; Co, 0.02 g; methionine, 1.2 g; cysteine, 0.8 g; lysine,1.3 g; arginine, 0.6 g; phenylalanine, 0.4 g; tryptophan, 0.7 g.

[b] Including some minor components not shown.

[c] Including other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18, C20 and C22.

[d] DHA/EPA, 22:6 n-3/ 20:5 n-3; EPA/ARA, 20:5 n-3/ 20:4n-6.

The ingredients, proximate and FA composition of the experimental diets are shown in Table 7.1. Briefly, the diet containing FO as the sole lipid source was used as the reference diet (FO100) whereas a blend of VO consisting of PO and LO (4:1) replaced 50% and 100% of the FO in the VO50 and VO100 diets, respectively. In all diets, 16:0 accounted for the bulk of saturated fatty acids (SFA), 18:1n-9 for monounsaturated fatty acids (MUFA), 18:2n-6 for n-6 PUFA, and EPA and DHA for n-3 LC-PUFA.

Moreover, gradual inclusion of the VO mixture increased dietary C16:0 and total SFA (30.2 to 36.1% of total FA), C18:1n-9 and total MUFA (22.4 to 29.3%), and C18:2n-6 and total n-6 PUFA (8.2 to 12.3%) while decreased EPA, DHA and total n-3 PUFA (32.4 to 19.1%) despite C18:3n-3 raised from 1.1 to 6.0% of total FA. DHA/EPA and EPA/ARA ratios remained unchanged among diets (Table 6.1).

2.3. Fish sampling and growth evaluation

Fish were anaesthetized with 10 mg/L clove oil containing 87% eugenol (Guinama®, Valencia, Spain) for individual weight and fork length measurements at the beginning, end, and regularly at 30 days-intervals after the start of the feeding trial. In addition, at the end of the experiment eight fish from each treatment were collected for blood, liver and muscle sampling. Blood was drawn via the ventral aorta using 5 mL heparinized syringes, centrifuged at 3000 g for 5 min at 4 ºC to separate the plasma which was stored at -30 ºC until further analyses. Next, the fish were euthanized with an overdose of clove oil

and portions of liver and dorsal muscle rapidly excised, frozen in liquid nitrogen and stored at -80 ºC for subsequent biochemical determinations.

The effect of dietary treatments on culture performance was determined by evaluating growth, survival and nutrient utilization indices, including weight gain (WG), specific growth rate (SGR), feed intake (FI) and feed conversion ratio (FCR) at the end of the feeding trial (Table 7.2).

All procedures were carried out in accordance to the European Directive 2010/63/EU and Spanish national legislation (Spanish Royal Decree 53/2013), which regulate animal usage in experimentation and/or other scientific purposes.

Table 7.2 Growth performance and feed utilization of *Seriola dumerili* juveniles fed the experimental diets for 154 days.

	FO100	**VO50**	**VO100**
Initial weight (g)	39.6 ± 3.7	37.9 ± 1.0	40.2 ± 2.9
Final weight (g)	390 ± 23.2	397 ± 24.4	375 ± 30.8
WG (%)[1]	894 ±96	940 ±75	840 ± 16
SGR (%/day)[2]	1.49 ±0.07	1.50 ± 0.05	1.47 ± 0.02
FI (g/100g fish day)[3]	1.82 ± 0.26	1.81 ± 0.13	1.79 ± 0.02
FCR[4]	1.75 ± 0.27	1.75 ± 0.14	1.72 ± 0.03
Survival (%)	75 ± 6	74 ± 6	74 ± 7

Data are expressed as mean ± SD
[1]Weight gain = 100 x [(final weight – initial weight) / initial weight];
[2]Specific growth rate = 100 x (ln final weight – ln initial weight) / feeding days;
[3]Feed intake = 100 x feed consumption (g) / average biomass (g) x days;
[4]Feed conversion ratio = dry food fed (g) / wet weight gain (g).

2.4. Analytical procedures

Plasma glucose concentration (mg/dL), activities of glutamate–oxalacetate transaminase GOT (AST) (EC 2.6.1.1) and glutamate–pyruvate transaminase GPT (ALT) (EC 2.6.1.2) (U/L 37 ºC) were determined by enzymatic kits according to the manufacturer's instructions (Human, Wiesbaden, Germany). One unit (U) of aminotransferases activity was defined as 1 µmol of NADH disappearance per minute. Concentrations of triglyceride (mg/dL) and cortisol (ng/mL) were measured with a diagnostic kit (Gernon, Barcelona, España) and an enzyme immunoassay kit (Arbor Assays, MI, USA), respectively. Lipase (E.C. 3.1.1) activity (U /L 30 ºC) was assayed by slight modifications of the method previously described by Gisbert et al., (2009) considering one unit of activity equivalent to 1 µmol of p-nitrophenol myristate hydrolyzed per min.

Proximate composition of the experimental diets and whole body fish were determined according to the following procedures: moisture by oven thermal drying at 110 ºC to constant weight, ash by combustion in a muffle at 550 ºC overnight, and crude protein (N x 6.25) by sample digestion using the Kjeldhal method. Quantification of crude fat was performed by ether extraction with an Ankom XT10 Extraction System (NY, USA) (AOCS, 2005). Energy was calculated according to Brouwer (1965), from the C (g) and N (g) balance (GE = 51.8 × C − 19.4 × N).

Liver and muscle total lipid (TL) was extracted by homogenization in chloroform/methanol (2:1, v/v) according to Folch et al., (1957). The organic solvent was evaporated under a stream of nitrogen, the lipid content gravimetrically determined (Christie, 1982) and stored in chloroform/methanol (2:1) containing 0.01% butylated hydroxytoluene (BHT) at -20 ºC until further analysis. The lipid extract was subjected to acid-catalyzed transmethylation with 1% sulphuric acid (v/v) in methanol, and the resultant fatty acid methyl esters (FAME) purified by thin layer chromatography (TLC) (Christie, 1982). During acid-catalyzed transmethylation, FAMEs are formed simultaneously with dimethyl acetals (DMAs) which originate from the 1-alkenyl chain of plasmalogens. FAME and DMA were separated and quantified on a TRACE-GC Ultra gas chromatograph (Thermo Scientific,

Milan, Italy) equipped with an on-column injector, a flame ionization detector and a fused silica capillary column, Supelcowax TM 10 (30 m x 0.32 mm x 0.25 µm film thickness) (Supelco Analytical, Bellefonte, PA, USA). Helium at a flow of 1.5 mL/min was used as the carrier gas. Individual FAME and DMA were identified by reference to authentic standards, and further confirmation of identity was carried out by mass spectrometry when necessary.

2.5 Indices of the nutritional quality of lipids

The influence of increasing levels of FO substitution on the nutritional quality of the fish fillet lipid fraction was monitored through indices based on the functional effects of its constituent FA. Equations were used to determine the index of atherogenicity (IA) (Ulbricht & Southgate, 1991), the index of thrombogenicity (IT) (Ulbricht & Southgate, 1991), and the flesh lipid quality (FLQ) (Abrami et al., 1992), respectively.

$$\mathrm{IA} = \frac{\mathrm{C12{:}0} + (4 \times \mathrm{C14{:}0}) + \mathrm{C16{:}0}}{\Sigma\,\mathrm{MUFA} + \mathrm{n6\ PUFA} + \mathrm{n3\ PUFA}}$$

$$\mathrm{IT} = \frac{\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0}}{(0.5 \times \Sigma\mathrm{MUFA} + 0.5 \times \mathrm{n6PUFA} + 3 \times \mathrm{n3PUFA}) + \left(\frac{\mathrm{n3\ PUFA}}{\mathrm{n6\ PUFA}}\right)}$$

$$\mathrm{FLQ} = \frac{\mathrm{C20{:}5n-3} + \mathrm{C22{:}6n-3}}{\Sigma\ \mathrm{total\ FA}}$$

Briefly, the two first indices indicate that C12:0, C14:0 and C16:0 are atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory systems), and that C14:0, C16:0 and C18:0 are thrombogenic, facilitating the formation of clots in the blood vessels. The third equation, reveals the percentage relationship in which the main n-3 LC-PUFA (EPA and DHA) appear in muscle with respect to the totality of the lipids.

2.6. Statistical analysis

Prior to analysis, all data expressed as percentage were arcsine-transformed. Normal distribution was checked with the Kolmogorov–Smirnoff test and homogeneity of variances by the Levene test. Comparisons among dietary groups (FO100, VO50 and VO100) were assessed by one-way ANOVA and significant differences identified by the Tukey post hoc test. When homoscedasticity and/or normality was not achieved, data were subjected to the non-parametric Kruskal–Wallis test followed by the Games–Howell test for post-hoc comparisons. Differences among means were accepted when $P < 0.05$. Statistical analyses were carried out using the SPSS package version 20.0 for Windows (SPSS, Inc., Chicago, IL, USA).

3. RESULTS

3.1. Growth performance and feed utilization

At the end of the feeding period, no negative effects were found with either the partial (50%) or complete FO substitution with the mixture of PO and LO (4:1) in growth and feed performance, although values were generally numerically inferior in fish fed the no FO-diet. Briefly, all dietary groups presented similar final body weight (390, 397 and 375 g for FO100, VO50 and VO100, respectively) which resulted in steady weight gains of 894, 940 and 840 g, respectively. All diets were readily accepted by the fish, with the mean daily FI being 1.81 g 100 g fish/day, and the average FCR, 1.74. Final survival rate was 75% for all dietary groups (Table 7.2).

3.2. Biometric parameters and body proximate composition

None of the somatic parameters studied (condition factor, viscerosomatic, hepatosomatic and mesenteric fat indices, ingested fat retention and ingested energy retention) significantly varied with increasing FO replacement (Table 7.3). Similarly, no trend in protein, lipid or ash of fish whole-body was apparent in dietary groups. Only

moisture content varied among treatments, being significantly lower in fish fed the control diet (FO100) than in those receiving VO50.

Table 7.3 Biometric indices and proximate composition of *Seriola dumerili* juveniles fed the experimental diets for 154 days.

	FO100	VO50	VO100
CF[1]	1.37 ± 0.17	1.30 ± 0.24	1.28 ± 0.10
VSI (%)[2]	4.32 ± 0.62	4.18 ± 1.10	4.33 ± 0.52
HSI (%)[3]	0.87 ± 0.20	0.78 ± 0.15	0.84 ± 0.10
MFI (%)[4]	0.18 ± 0.17	0.05 ± 0.15	0.10 ± 0.09
IFR (%)[5]	38.4 ± 4.17	34.1 ± 3.23	34.6 ± 4.60
IER (%)[6]	21.7 ± 0.91	20.6 ± 1.12	21.4 ± 0.83
Whole body proximate composition g/kg (ww)			
Moisture	696.5 ± 0.9[a]	706.3 ±0.9[b]	702.5 ± 0.2[ab]
Crude protein	192.2 ± 1.3	189.1 ± 1.5	188.7 ± 2.3
Total lipid	77.8 ± 2.9	72.9 ± 2.0	74.7 ± 1.6
Ash	28.1 ± 0.9	27.6 ± 0.3	26.9 ± 0.8

ww, wet weight; Data are expressed as mean ± SD. Means with different superscripts letters are significantly different ($P < 0.05$).

[1] Condition factor = 100 x (final weight / total length^3);

[2] Viscerosomatic index = 100 x (viscera weight / final weight);

[3] Hepatosomatic index = 100 x (liver weight / final weight);

[4] Mesenteric fat index = 100 x (viscera fat / final weight);

[5] Ingested fat retention = 100 x (fish fat gain / crude fat intake);

[6] Ingested energy retention = 100 x (fish energy gain, kJ/ gross energy intake, kJ).

3.3. Plasma biochemical determinations

As it is shown in Table 7.4, glucose, triglycerides and cortisol concentrations remained fairly constant among treatments (186-223 mg/dL, 89-98 mg/dL, and 54-56 ng/mL, respectively). Likewise, the activities of the enzymes GOT (10.9-20.6 U/L), GPT (3.9-6.1 U/L) and lipase (7.1-7.8 U/L) were not affected by the diet.

Table 7.4 Plasma parameters of greater amberjack juveniles fed the experimental diets for 154 days.

	FO100	VO50	VO100
Glucose (mg/dL)	223.4 ± 48.4	190.9 ± 35.5	1859 ± 55.5
GOT (U/L)	20.6 ± 11.5	12.6 ± 6.2	10.9 ± 0.8
GPT (U/L)	6.1 ± 1.6	4.9 ± 1.2	3.9 ± 1.4
Triglycerides (mg/dL)	94.4 ± 18.6	89.0 ± 25.8	97.8 ± 28.8
Cortisol (ng/mL)	54.2 ± 1.0	56.1 ± 16.3	55.2 ± 6.1
Lipase (U/L)	7.8 ± 1.0	7.6 ± 0.3	7.1 ± 0.6

Data are expressed as mean ± SD (n=6).

3.4. Tissue biochemical composition

The TL contents of liver and muscle did not vary among treatments, neither when compared to the initial sample, although the liver presented significantly higher values than muscle ranging from 7.9 to 8.8 % of fresh weight, and 0.7 to 0.9 % of fresh weight, respectively (Figure 7.1). Both tissues followed similar patterns of FA profiles and variations with respect to the initial sample in response to increasing FO substitution (Tables 7.5 and 7.6, respectively). Briefly, despite the relative proportion of C16:0 was higher in fish fed the no FO diet (VO100), no significant variations among treatments existed in the total percentage of SFA. Total MUFA raised significantly with higher VO inclusion whereas total PUFA, n-6 and n-3 LC-PUFA showed the opposite trend. Individually, C18:1n-9 (which represented 50-80% of total MUFA), C18:2n-6 and C18:3n-3 were higher when complete FO substitution, whereas ARA, EPA, C22:5n-3 (DPA, docosapentaenoic acid) and DHA, reached higher values in fish fed the 100% FO-diet. Hepatic DHA/EPA ratio increased and EPA/ARA ratio decreased with reduced dietary FO (Table 7.5), which, conversely, remained unchanged in muscle (Table 7.6).

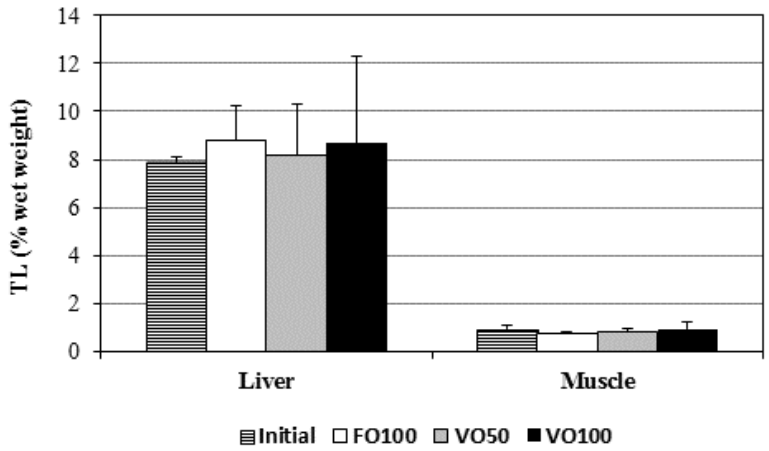

Figure 7.1 Total lipid content (% ww) of liver and muscle of *Seriola dumerili* juveniles feed the experimental diets for 154 days. The bars represent the mean of N replicates plus minus SD.

Muscle and liver showed a tissue-specific fatty acid profile, with muscle containing lower proportions of MUFA, and higher PUFA, n-3 and n-6 LC-PUFA than liver. DMA were present exclusively in muscle (2.5 to 3.0% of total FA). Irrespective to diet, C18 MUFA and C18:2n-6 proportions were 1.5 to 2-fold lower in muscle than in the liver (5.8, 9.4 and 14.0 vs 11.2, 19.8 and 26.8; 4.8, 7.2 and 10.7 vs 9.7, 12.4 and 15.3, respectively), whereas C22:6n-3 was 3 to 4-fold higher in the muscle (29.6, 26.7 and 22.0 vs 9.9, 7.2 and 5.5% of total FA, respectively).

Table 7.5 Total FA (µg/mg DM) and main fatty acid composition (% total fatty acids) of liver TL from cultured *Seriola dumerili* juveniles fed the experimental diets for 154 days.

	Initial sample	**FO100**	**VO50**	**VO100**
Total FA	221.74 ± 29.09	191.63 ± 20.2	222.07 ± 16.3	186.53 ± 24.6
Total SFA[1]	30.31 ± 1.00	30.25 ± 0.89	31.54 ± 0.86	31.28 ± 2.50
14:0	4.96 ± 0.18	4.62 ± 0.23^{c}	2.95 ± 0.12^{b}	1.69 ± 0.10^{a}
16:0	20.52 ± 0.87	20.33 ± 0.76^{a}	22.71 ± 1.03^{b}	23.85 ± 2.30^{b}
18:0	3.63 ± 0.16	4.24 ± 0.45	5.04 ± 0.47	4.99 ± 0.61
Total MUFA[1]	28.39 ± 1.62	24.92 ± 1.03^{a}	30.52 ± 1.32^{b}	33.86 ± 1.68^{b}
16:1[2]	9.27 ± 0.18	7.71 ± 0.06^{c}	5.39 ± 0.05^{b}	3.22 ± 0.09^{a}
18:1[2]	18.08 ± 1.14	15.89 ± 1.22^{a}	23.92 ± 0.92^{b}	29.73 ± 1.56^{c}
20:1[2]	0.61 ± 0.18	0.55 ± 0.06	0.52 ± 0.08	0.54 ± 0.16
Total PUFA[1]	38.16 ± 2.65	43.92 ± 0.90^{b}	37.37 ± 1.13^{a}	34.48 ± 1.81^{a}
18:2 n6	8.80 ± 0.36	9.70 ± 0.31^{a}	12.40 ± 0.72^{b}	15.31 ± 1.09^{c}
18:3 n3	0.93 ± 0.04	1.23 ± 0.11^{a}	3.25 ± 0.03^{b}	5.29 ± 0.66^{c}
18:4 n3	1.29 ± 0.16	1.32 ± 0.14^{c}	0.73 ± 0.03^{b}	0.29 ± 0.05^{a}
Total n6LC-PUFA[1]	1.56 ± 0.06	1.96 ± 0.16^{c}	1.47 ± 0.06^{b}	1.13 ± 0.09^{a}
20:2 n6	nd	0.26 ± 0.05	0.29 ± 0.05	0.31 ± 0.09
20:4 n6	1.26 ± 0.05	1.28 ± 0.08^{c}	0.92 ± 0.03^{b}	0.61 ± 0.09^{a}
22:5 n6	0.29 ± 0.02	0.29 ± 0.01^{b}	0.20 ± 0.03^{a}	0.18 ± 0.04^{a}
Total n3LC-PUFA[1]	23.77 ± 2.65	27.75 ± 0.87^{c}	18.50 ± 0.71^{b}	12.03 ± 1.04^{a}
20:4 n3	0.76 ± 0.01	0.94 ± 0.12^{c}	0.67 ± 0.06^{b}	0.34 ± 0.05^{a}
20:5 n3	11.50 ± 1.03	13.48 ± 0.31^{c}	8.07 ± 0.31^{b}	4.59 ± 0.30^{a}
21:5 n3	0.39 ± 0.03	0.46 ± 0.01^{c}	0.31 ± 0.01^{b}	0.16 ± 0.01^{a}
22:5 n3	2.37 ± 0.13	2.92 ± 0.35^{c}	2.28 ± 0.22^{b}	1.43 ± 0.06^{a}
22:6 n3	8.75 ± 1.46	9.95 ± 1.12^{b}	7.18 ± 0.89^{a}	5.36 ± 1.19^{a}
Ratios				
PUFA/SFA	1.26 ± 0.13	1.45 ± 0.04^{b}	1.19 ± 0.03^{a}	1.1 ± 0.13^{a}
N6/n3	0.40 ± 0.05	0.39 ± 0.02^{a}	0.62 ± 0.06^{b}	0.93 ± 0.06^{c}
DHA/EPA[3]	0.76 ± 0.06	0.74 ± 0.07^{a}	0.88 ± 0.04^{ab}	1.17 ± 0.23^{b}
EPA/ARA[3]	9.10 ± 0.53	10.53 ± 0.66^{b}	8.79 ± 0.42^{ab}	7.53 ± 1.23^{a}

Results are expressed as means ± SD (n=6-8) except for the initial sample where n=3. Means with different superscript letters indicate significant differences ($P < 0.05$).

1 Includes some minor components not shown.

2 Includes other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18 and C20.

3 DHA/EPA, 22:6 n-3/ 20:5 n-3; EPA/ARA, 20:5 n-3/ 20:4 n-6.

Table 7.6 Total FA (μg/mg DM) and main fatty acid composition (% total fatty acids) of muscle TL, and indices of nutritional quality of lipids from cultured *Seriola dumerili* juveniles fed the experimental diets for 154 days.

	Initial sample	FO100	VO50	VO100
Total FA	33.04 ± 6.45	16.75 ± 2.47	20.31 ± 4.93	23.94 ± 6.34
Total SFA[1]	28.44 ± 1.70	34.28 ± 0.28	33.64 ± 0.76	33.36 ± 0.47
14:0	2.47 ± 0.38	1.09 ± 0.29	0.95 ± 0.26	0.69 ± 0.13
16:0	17.79 ± 1.06	20.51 ± 0.17^{a}	20.44 ± 0.05^{a}	21.07 ± 0.12^{b}
18:0	7.27 ± 0.61	9.36 ± 0.22	9.38 ± 0.71	8.76 ± 0.37
Total MUFA[1]	20.22 ± 0.14	12.92 ± 1.14^{a}	16.15 ± 2.20^{ab}	19.24 ± 2.32^{b}
16:1[2]	5.27 ± 0.50	2.67 ± 0.48^{b}	2.22 ± 0.44^{ab}	1.73 ± 0.27^{a}
18:1[2]	13.31 ± 0.44	9.19 ± 0.87^{a}	12.71 ± 1.79^{ab}	16.46 ± 2.15^{b}
20:1[2]	0.76 ± 0.14	0.33 ± 0.06	0.32 ± 0.04	0.31 ± 0.04
Total PUFA[1]	49.20 ± 0.13	51.71 ± 1.42^{b}	49.83 ± 1.61^{ab}	47.13 ± 1.90^{a}
18:2 n6	5.08 ± 0.11	4.70 ± 0.030^{a}	7.14 ± 0.52^{b}	10.69 ± 0.32^{c}
18:3 n3	0.65 ± 0.04	0.38 ± 0.01^{a}	1.38 ± 0.29^{b}	2.59 ± 0.31^{c}
18:4 n3	0.91 ± 0.16	0.39 ± 0.08^{b}	0.30 ± 0.07^{b}	0.18 ± 0.04^{a}
Total n6LC-PUFA[1]	2.01 ± 0.06	2.60 ± 0.12^{b}	2.37 ± 0.25^{ab}	2.02 ± 0.25^{a}
20:2 n6	nd	0.14 ± 0.01^{a}	0.18 ± 0.01^{b}	0.19 ± 0.01^{b}
20:4 n6	1.43 ± 0.05	1.65 ± 0.08^{b}	1.44 ± 0.15^{ab}	1.18 ± 0.17^{a}
22:5 n6	0.58 ± 0.03	0.72 ± 0.06	0.72 ± 0.08	0.64 ± 0.07
Total n3LC-PUFA[1]	39.29 ± 2.06	43.08 ± 1.80^{c}	38.26 ± 2.34^{b}	31.45 ± 2.34^{a}
20:4 n3	0.52 ± 0.11	0.29 ± 0.03^{b}	0.30 ± 0.04^{b}	0.20 ± 0.02^{a}
20:5 n3	10.73 ± 1.29	9.78 ± 0.43^{c}	8.02 ± 0.24^{b}	6.40 ± 0.50^{a}
21:5 n3	0.35 ± 0.04	0.19 ± 0.03^{b}	0.20 ± 0.04^{b}	0.14 ± 0.02^{a}
22:5 n3	3.05 ± 0.04	2.87 ± 0.07^{b}	2.87 ± 0.08^{b}	2.47 ± 0.07^{a}
22:6 n3	24.64 ± 1.55	29.65 ± 2.33^{b}	26.86 ± 2.88^{ab}	22.23 ± 2.06^{a}
Total DMA[1]	1.02 ± 0.11	2.91 ± 0.04	2.77 ± 0.35	2.57 ± 0.46
16:0 DMA	0.63 ± 0.03	1.50 ± 0.08	1.48 ± 0.17	1.45 ± 0.31
18:0 DMA	0.39 ± 0.11	0.79 ± 0.05^{b}	0.72 ± 0.07^{ab}	0.63 ± 0.06^{a}
Ratios				
PUFA/SFA	1.74 ± 0.17	1.51 ± 0.04^{b}	1.48 ± 0.03^{ab}	1.41± 0.04^{a}
N6/n3	0.17 ± 0.01	0.17 ± 0.01^{a}	0.24 ± 0.02^{b}	0.37 ± 0.02^{c}
DHA/EPA[3]	2.32 ± 0.33	3.02± 0.34	3.34 ± 0.19	3.46 ± 0.12
EPA/ARA[3]	7.48 ± 0.70	5.93 ± 0.19	5.56 ± 0.51	5.42 ± 0.39
IA		0.40 ± 0.02	0.39 ± 0.03	0.38 ± 0.02
IT		0.21 ± 0.01^{a}	0.22 ± 0.01^{a}	0.25 ± 0.01^{b}
FLQ		39.39 ± 1.83^{b}	34.88 ± 3.09^{b}	28.63 ± 2.89^{a}

Results are expressed as means ± SD (n=8), except for the initial sample where n=3. Means with different superscript letters indicate significant differences ($P < 0.05$).

[1] Includes some minor components not shown.

[2] Includes other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18 and C20.

[3] DHA/EPA, 22:6 n-3/ 20:5 n-3; EPA/ARA, 20:5 n-3/ 20:4 n-6.

3.5 Indices of the nutritional quality of lipids

The indices used to assess the nutritional value of the flesh lipid fraction are shown in Table 6.6. Both PUFA/SFA and n-6/n-3 ratios were more favorable in terms of nutritional value in fish fed the diet with FO as the unique lipid source, decreasing with higher inclusion of the VO mixture. IA remained unchanged irrespective of dietary FO substitution (0.42-0.43) whereas complete FO replacement promoted a significant increase in IT (0.23 ± 0.01) compared to FO100 and VO50-fed fish (0.20 ± 0.01 and 0.21 ± 0.01, respectively). Finally, FLQ decreased with gradual FO replacement (39.6 ± 2.0, 34.7 ± 4.0 and 28.3 ± 4.3, respectively).

4. DISCUSSION

In the present study, the plant-based oil mixture consisting of PO and LO (4:1) used to partially (50%) or totally substitute FO did not significantly affect greater amberjack, *S. dumerili* growth performance and feed efficiency (Table 7.2). Both SGR and FCR of fish receiving the VO-based blend are similar or even improve most values reported for fish of the same size class cultured in PVC tanks or floating cages fed fish scraps or FO-based diets in the western Mediterranean coast (reviewed by Mazzola et al., (2000).

A number of previous studies have reported that a large fraction (60-70 %) of dietary FO may be replaced by VO blends without compromising fish production (Benedito-Palos et al., 2008; Fountoulaki et al., 2009; Izquierdo et al., 2003; Menoyo et al., 2004; Mourente & Bell, 2005; Peng et al., 2008). However, some species are negatively affected by total substitution of FO (Nasopoulou & Zabetakis, 2012; Regost et al., 2003; Sales & Glencross, 2011) while other reports show not effect (Glencross et al., 2016; Mozanzadeh et al., 2016) so it becames necessary to study carefully FO substitution effects for any particular fish species. Big pelagic marine carnivorous fish species such as *S. quinqueradiata* did not vary growth performance when receiving diets with increasing olive oil inclusion to completely replace FO (Seno-O et al., 2008) in a short-term feeding

trial of 40 days. On the contrary, both cobia (*Rachycentron canadum*) and yellowtail kingfish (*S. lalandi*) juveniles production performance was compromised when FO was totally substituted by sunflower or canola oil, respectively (Bowyer et al., 2012; Trushenski et al., 2011). Overall, successful fish performance may be achieved when FO sparing with alternative oils of terrestrial origin as long as their minimum EFA requirements are met. In our work, FO100, VO50 and VO100 diets provide 2.7, 2.1 and 1.2% n-3 LC-PUFA of dry matter respectively, which is sufficient to cover the EFA requirements for most marine fish species (Glencross, 2009; Tocher, 2010). Consequently, although *S. dumerili* nutritional requirements are still unknown and the EFA requirements vary qualitatively and quantitatively with both species and growth stage, it seems that formulation with 525 g/kg of FM contributes to supply enough LC-PUFA to meet fish needs even in the absence of FO, since FM usually contains up to 8-15% of crude lipid, with a 30-35 % of n-3 LC-PUFA (Bimbo, 2000). In fact, our present results seem to indicate that the EFA requirements of greater amberjack juveniles may be met by levels of n-3 LC-PUFA up to 1.2 % of the dry weight of the diet. As far as we know, this is the first reference on the quantitative EFA requirements for this species.

Regardless of whether FO replacement affects fish growth and feed performance, its impact on tissue lipid deposition and fatty acid composition is controversial, varying depending on the species, dietary lipid content and substitute lipid source (Turchini et al., 2009). Previous research suggest that SFA and MUFA-rich lipid diets can make LC-PUFA utilization and/or diet-to-tissue transfer more efficient (Bowzer et al., 2016; Pérez et al., 2014; Turchini et al., 2009). The PO:LO (4:1) mixture used here seem to provide balanced proportions of SFA: MUFA: PUFA and n-6/n-3 ratio for maintaining or even improving DHA/EPA and EPA/ARA ratios in muscle (3.3 and 5.6 for VO50; 3.5 and 5.7, for VO100, respectively) with respect to the initial fish (2.32 and 7.48) and fish receiving the 100% FO diet (3.0 and 5.8, respectively). The same tendency for both proportions was observed in the liver of VO-fed groups (0.76 and 9.10; 0.74 and 10.58; 0.88 and 8.81; 1.20 and 7.68; for the initial, FO100, VO50 and VO100 fish, respectively). In addition, physiologically important DHA/EPA and

EPA/ARA ratios obtained in our present work are similar to those previously reported for farmed greater amberjack adults and similar to wild counterparts (Rodríguez-Barreto et al., 2012; Saito, 2012).

The liver is the major site of lipid storage in the majority of marine fish species being commonly used as indicator of unsuitable dietary fat ingestion. The diagnosis of healthy liver should allow optimized diets to be devised for a given species. It is well established that replacing dietary FO by terrestrial oils may produce the accumulation of fat in fish liver giving rise to a fatty liver syndrome (Benedito-Palos et al., 2008; Díaz-López et al., 2010; Piedecausa et al., 2007; Sargent et al., 2002), which may be associated with increased lipid peroxidation and impaired function such as inefficient nutrient utilization and necrosis (Craig et al., 1999; Tucker et al., 1997). In our study, both the liver fat content and the HSI of VO50 and VO100-fed fish were similar to the control and initial fish, suggesting no hepatic affection with increasing levels of PO:LO inclusion. These observations agree well with previous research on turbot (*Psetta maxima*) (Regost et al., 2003), European seabass (Richard et al., 2006) and gilthead sea bream (Bouraoui et al., 2011) where no impairment of lipogenic activity and lipid content in fish liver was detected when using PO and/or LO to replace FO. In line with this, Lemaire et al., (1991) found correlations between plasma biochemical parameters and hepatic histopathological condition. Thus, plasma parameters are often regarded as suitable monitoring tools of the physiological status of the fish (Bowyer et al., 2012; Coz-Rakovac et al., 2008; Díaz-López et al., 2009; Kowalska et al., 2012) and could also be used as physiological indicators of lipogenesis affection with FO substitution (Richard et al., 2006). Under our experimental conditions, the inclusion of PO and LO did not affect plasma chemistry suggesting that fish were in acceptable nutritional status adding more evidences to the proper hepatic functioning even under FO absence. However, the higher relative content of C18:1n-9 and C18:2n-6, along with lower proportions of LC-PUFA, especially ARA, EPA and DHA, in the liver of VO-fed fish might have a long-term detrimental impact on lipid/lipoprotein metabolism, since they have been reported to modulate lipid metabolism at different levels reviewed by Turchini et al., (2009). Thus, longer-term studies are needed to rule out possible

hepatic damage caused by the PO:LO mixture not detected in the present 5 months-feeding trial.

Regardless of dietary inputs, muscle displayed higher relative content of n-3 LC-PUFA than the liver or diet. This indicates that LC-PUFA, particularly DHA, are selectively retained in greater amberjack fillets, as previously reported in salmon (Bell et al., 2001; Bell & Sargent, 2003; Torstensen et al., 2004), and other marine fish species (Bowyer et al., 2012; Mourente & Bell, 2005; Pérez et al., 2014). The high supply of SFA, especially C14:0 and C16:0, and MUFA chiefly C18:1n-9, in VO50 and VO100 diets may have promoted their preferential use as metabolic energy for swimming (Bell, et al., 2003; McKenzie, 2001; Stubhaug et al., 2007; Torstensen et al., 2004) enhancing muscle deposition of LC-PUFA.

There is currently increasing interest on the intake of marine-based feedstuff for its health-promoting benefits to humans. Several FA ratios and indices have been defined to assess the nutritional quality of food lipid for human consumption. According to nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45 (Wood et al., 2004) and, within the PUFA, a ratio of 1:1 to 2:1 n-6/n-3 should be the target ratio for health (Simopoulos, 2011). Lower ratios of PUFA/SFA in the diet may increase the incidence of cardiovascular disease (WHO, 2003). Further, fats with lower indices of atherogenicity (IA) and thrombogenicity (IT) can inhibit the aggregation of platelets and decrease the levels of esterified FA, cholesterol and phospholipids, thereby preventing the appearance of micro and macrocoronary diseases (Turan et al., 2007). The indices of lipid quality selected in the present work clearly indicate that flesh from greater amberjack juveniles is a nutritionally adequate food for human consumption although the gradual inclusion of the PO:LO mixture tended to partially reduce its value. In brief, and regardless of dietary treatment, both PUFA/SFA and n-6/n-3 are well within values recommended for healthy human. Although there is no recommended values for IA and IT, it is generally accepted that the lower the values the healthier the ratios. So, the low values of both IA and IT indices together with high FLQ present in flesh suggest that its consumption may help to prevent the development of coronary heart diseases,

being more favorable in terms of lipid quality for human consumption than gilthead seabream or European seabass (Grigorakis, 2007; Pérez et al., 2014).

In summary, the present work provides valuable information to the successful and economically viable culture of greater amberjack. The mixture of PO and LO (4:1) can effectively replace completely dietary FO in FM-based diets for *S. dumerili* juveniles without affecting growth performance, feed utilization and fish health. Based on these results, it appeared that a 1.2% of EFA in a dry weight basis may cover the EFA requirements for juveniles of this species. In terms of product quality, and regardless of dietary lipid, flesh of cultured specimens displayed good nutritional and healthy characteristics for human consumption, in line with current global guidelines for fat intake.

5. ACKNOWLEDGMENTS

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Capítulo 8
DISCUSIÓN GENERAL

1. Digestibilidad de los ingredientes evaluados

La prueba de digestibilidad realizada permitió formular los piensos de los ensayos posteriores, con los niveles digestibles de manera que cubrieran las necesidades nutritivas de la especie.

La digestibilidad proteica obtenida (Tabla 8.1) osciló entre el 75,8 y el 91,3% en el caso de los ingredientes de origen animal y el 48,5 y el 87% en los de origen vegetal, lo que nos confirma una alta digestibilidad de los productos de origen animal en un pez carnívoro marino como es el caso de la *Seriola dumerili* (Tibbetts et al., 2006), al igual que ocurre en otras especies como la dorada y la lubina, en las que experimentos similares obtuvieron resultados de digestibilidad en ingredientes de origen animal entre el 80 y el 90% (Gomes da Silva & Oliva-Teles, 1998; Lupatsch et al., 1997).

En cuanto a la digestibilidad de los lípidos, ambos tipos de ingredientes, animales y vegetales, presentaron unos buenos datos de digestibilidad, entre un 83,5% y un 94%, existiendo solo pequeñas diferencias entre ellos; estos resultados son similares a los obtenidos en otras especies como dorada, en la que los lípidos presentaron unos datos de digestibilidad entre 85 y 95% (Lupatsch et al., 1997).

Económicamente, las materias primas vegetales presentan un precio similar entre ellas (Tabla 8.1), excepto el gluten de trigo que tiene el precio más elevado. Sin embargo, respecto a las materias primas animales existen mayores diferencias en relación con el precio de estas, según su nivel de producción y de su utilización como subproducto, así, los precios más elevados son losde la harina de krill y la harina de calamar (superiores a 2€/kg en ambos casos), coste incluso superior al de la harina de pescado, por lo tanto, la relación calidad-precio no es buena. Por otro lado, tanto la harina de pollo como la harina de krill desengrasado, al ser subproductos, presentan un precio por kilogramo inferior a 0,2€/kg, casi 8 veces menor que la harina de pescado (1,51€/kg), lo que sumado a su alta digestibilidad proteica las hace muy buenas candidatas como ingredientes proteicos en piensos para seriola.

Para relacionar el coste de los ingredientes y la digestibilidad proteica de los mismos, se calculó el precio de la proteína digestible (Tabla 8.1) para cada materia prima analizada, utilizando la siguiente fórmula:

$$Precio\ prot.digestible\ = \frac{Precio_{mat\ prima}}{\frac{Proteína_{mat\ prima}}{100} * \frac{CDA_{prot}}{100}}$$

Tabla 8.1 Precios de la proteína digestible de los diferentes ingredientes utilizados en la prueba de digestibilidad.

Ingredientes	Precio (€/kg)	Proteína (g/kg)	CDA_{ms}	CDA_p	Precio proteína digestible (€/kg)	Precio mat. seca digestible (€/kg)
Harina de haba	0,26	237	42,2	54,9	2,02	0,70
Harina de camelina	0,35	391	58,8	48,5	1,82	0,64
Harina de soja	0,32	499	54,9	73,2	0,87	0,66
Harina de guisante	0,22	216	57,2	63,0	1,59	0,43
Harina de girasol	0,33	291	65,7	62,9	1,79	0,56
Gluten de trigo*	**0,76**	**810**	**82,2**	**87,0**	**1,08**	**0,99**
Harina de krill desengrasado*	**0,25**	**723**	**68,0**	**86,7**	**0,40**	**0,42**
Harina de krill	2,30	713	87,2	91,3	5,41	2,92
Harina de pescado*	**1,51**	**561**	**57,7**	**75,8**	**2,32**	**2,95**
Harina de calamar	2,05	531	70,2	85,0	3,34	0,29
Harina de pollo*	**0,20**	**719**	**70,1**	**85,2**	**0,44**	**3,15**

CDA_{ms}: Coeficiente de digestibilidad aparente de la materia seca; CDA_p: Coeficiente de digestibilidad aparente de la proteína. El precio de la mat seca digestible se ha calculado de la misma manera.

*Los ingredientes señalados fueron los seleccionados para las pruebas posteriores.

Algunas de las fuentes vegetales como la harina de haba y la harina de camelina fueron descartados por su baja digestibilidad proteica (54,9 y 48,5%, respectivamente).

2. Evaluación de los parámetros productivos

Los datos de crecimiento y parámetros nutritivos obtenidos a lo largo de los distintos experimentos aparecen resumidos en la siguiente tabla (Tabla 8.2).

Tabla 8.2 Crecimiento y parámetros nutritivos en *Seriola dumerili* alimentada con piensos con distintas sustituciones de harina o aceite de pescado.

	Pienso	$P_{inicial}$	P_{final}	S	TCI	ICA	CTC
Sustitución Proteica I (Cap. 5)	FM100	39,6	385	75[a]	1,50	1,77	$3,9*10^{-3}$
	FM66	38,1	391	86[a]	1,51	1,68	$3,9*10^{-3}$
	FM33	38,1	348	86[a]	1,43	1,74	$3,6*10^{-3}$
	FM0	36,7	333	23[b]	1,40	1,80	$3,5*10^{-3}$
Sustitución Proteica II (Cap. 7)	FM100	525,4	853,1[a]		0.56[a]	1,55	$1,8*10^{-3}$ [a]
	FM66	517,1	854,8[a]		0,56[a]	1,45	$1,7*10^{-3}$ [a]
	FM33	547,1	750,8[b]		0,41[b]	1,65	$1,2*10^{-3}$ [b]
Sustitución Lipídica (Cap. 6)	FO100	39,6	385	75	1,50	1,77	$3,3*10^{-3}$
	VO50	37,9	397	74	1,5	1,75	$3,5*10^{-3}$
	VO100	40,2	375	74	1,47	1,72	3,3*10-3

Donde $P_{inicial}$: Peso inicial; P_{final}: Peso final; S: supervivencia; TCI: tasa de crecimiento instantáneo; ICA: índice de conversión del alimento, CTC: coeficiente térmico de crecimiento. Se muestra en verde el tratamiento que más interesa (máxima sustitución sin perjudicar el crecimiento) y en rojo, el que menos.

En la tabla 8.2 se muestra el valor del coeficiente térmico de crecimiento (CTC) de los distintos experimentos de la presente tesis. En este caso, la seriola presentó unos valores de CTC muy altos (hasta $3,9*10^{-3}$) si se comparan con otras especies mediterráneas, como la dorada, lubina o rodaballo, los cuales presentan unos valores medios de $1*10^{-3}$; $0,85*10^{-3}$ o $0,99*10^{-3,}$ respectivamente (Alamar et al., 2016; Kaushik, 1998). Con este valor de CTC en *S. dumerili*, se confirma, como se ha comentado anteriormente, que las especies de este género presentan un rápido crecimiento (Avilés-Quevedo & Castelló-Orvay, 2004), muy superior al de la lubina, dorada o rodaballo (Muraccioli et al., 2000), lo que puede ofrecer una clara ventaja para su producción a nivel comercial.

2.1. Sustitución de la harina de pescado

En la tabla 8.3 se recogen los resultados obtenidos en experimentos anteriores llevados a cabo en *S. dumerili* con distintas sustituciones de harina de pescado por fuentes proteicas alternativas, concretamente, harina de soja (Dawood et al., 2015; Tomás et al., 2005) y harina de pollo (Takakuwa et al., 2006a). El nivel máximo de sustitución que se consiguió sin que el crecimiento se viera afectado fue del 20-30%, sustituciones muy inferiores al las de la presente tesis doctoral, donde la utilización de una combinación de fuentes proteicas animales y vegetales ha permitido un porcentaje de sustitución en las dietas de hasta un 66%, y de un 33% en el caso de un periodo más largo de tiempo (capítulo 6). Por lo tanto, como se ha demostrado en experimentos previos (Sánchez-Lozano et al., 2009) la combinación de diversas proteínas alternativas para sustituir la harina de pescado puede alcanzar mayores niveles de sustitución, además de incluir una menor cantidad de aminoácidos sintéticos en la dieta por el efecto complentario de las proteínas alternativas.

Tabla 8.3 Resultados obtenidos en experimentos previos en *Seriola dumerili* alimentadas con distintas sustituciones de harina de pescado en distintas proporciones.

	% Sustitucion	$P_{inicial}$	P_{final}	S	TCI	ICA
Tomás et al., 2005.	20	450	980^{a}	41	$0,51^{a}$	$2,79^{a}$
	30	453	925^{a}	76	$0,46^{ab}$	$3,09^{a}$
	40	449	795^{b}	65	$0,38^{b}$	$4,57^{b}$
	50	447	670^{c}	48	$0,26^{c}$	$6,52^{c}$
Takakuwa et al., 2006.	0	93	178^{a}	97,5	1,27	$1,66^{a}$
	20	93	164^{abc}	97,5	1,14	$1,88^{ab}$
	40	93	149^{cb}	97,5	1,05	$2,22^{b}$
	60	93	146^{c}	97,5	1,07	$2,13^{b}$
Dawood et al., 2015.	0	25	$136,6^{b}$	100	$3,03^{b}$	1,07
	15	25	$136,0^{b}$	95	$3,02^{b}$	1,06
	30	25	$136,9^{b}$	100	$3,04^{b}$	1,06
	45	25	$101,7^{a}$	90	$2,49^{a}$	1,15

Donde $P_{inicial}$: Peso inicial; P_{final}: Peso final; S: supervivencia; TCI: tasa de crecimiento instantáneo; ICA: índice de conversión del alimento, CTC: coeficiente térmico de crecimiento. En rojo los peores crecimientos y en verde, el mejor.

En la segunda prueba, el crecimiento de los peces alimentados con el pienso con un 66% de sustitución se vio afectado, pudiendo deberse al largo periodo de alimentación con la misma dieta de alta sustitución (154 días del primer experimento, el periodo entre pruebas y 84 días del segundo), como ya se ha comprobado en trabajos anteriores, donde ingredientes animales y vegetales pueden incrementar el riesgo de desórdenes alimenticios, incluyendo cólico intestinal (bacalao) o dilatación gástrica (trucha) llegando a reducir el crecimiento a largo plazo (Baeverfjord et al., 2006; Estruch et al., 2018).

En relación con los resultados de supervivencia obtenidos, se puede ver una drástica reducción de la misma en peces alimentados con los piensos con una alta sustitución con fuentes proteicas alternativas. No presentaron signos de enfermedad, por lo que esta alta mortalidad puede estar ocasionada por el largo periodo de alimentación con piensos con fuentes proteicas vegetales, como ha sido observado en otras especies como la dorada (Estruch et al., 2015).

En la siguiente gráfica (Figura 8.1) se muestran las curvas de crecimiento de la *Seriola dumerili* alimentada con fuentes proteicas alternativas obtenidas en la presente tesis (Capítulo 5), así como las de otras especies mediterráneas que se producen en acuicultura, como la dorada o la lubina en ensayos también de sustitución de la harina de pescado.: tres experimentos realizados en lubina (Adamidou et al., 2009; Kaushik et al., 2004; Tibaldi et al., 2006) y cuatro realizados en dorada (Martínez-Llorens et al., 2009; Monge-Ortiz et al., 2016; Sánchez-Lozano et al., 2009, 2011). En todos ellos se realizaron sustituciones de harina de pescado por fuentes proteicas vegetales en porcentajes del 40 al 60%.

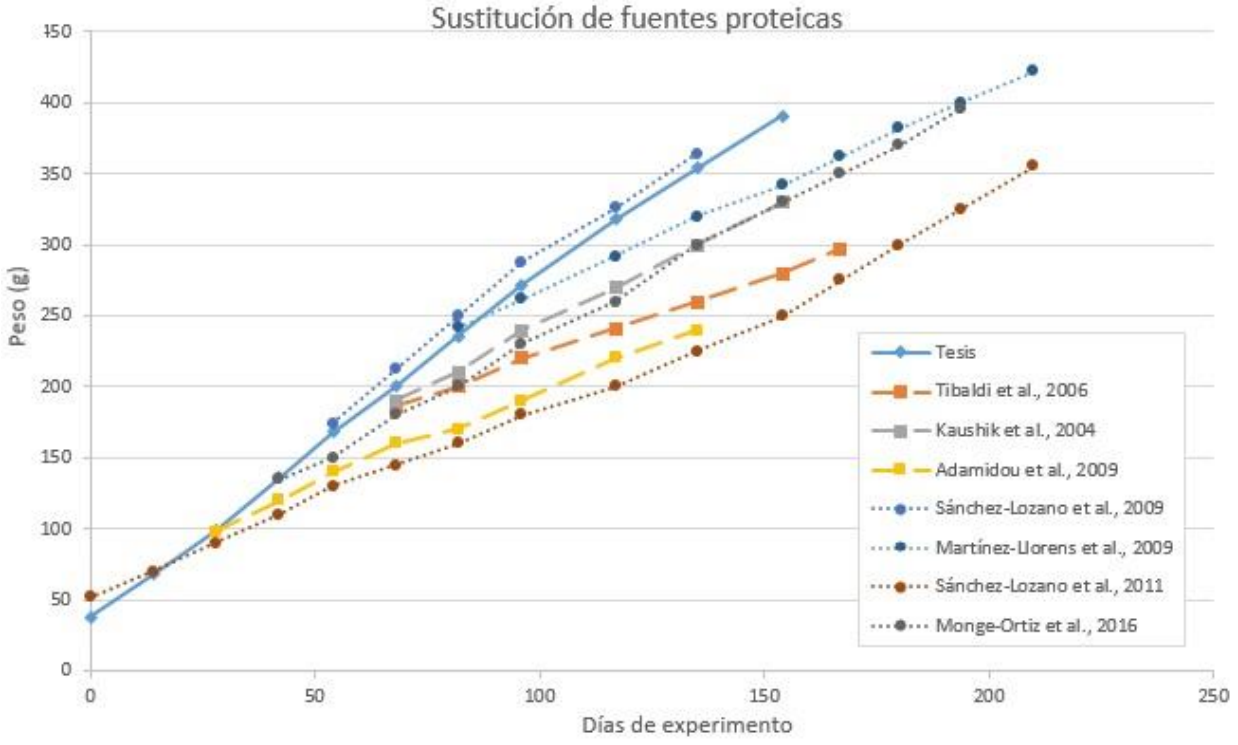

Figura 8.1 Evolución del peso medio de la seriola durante la presente tesis, y dorada y lubina (otros autores), alimentadas con piensos con fuentes proteicas alternativas. (--- : experimentos realizados en lubina; ····· : experimentos realizados en dorada).

Sánchez-Lozano et al. (2009) obtuvieron resultados de crecimiento similares a los del presente experimento en doradas alimentadas con concentrados proteicos de guisante y arroz hasta un 60% de sustitución, pero en el resto de los casos, se puede apreciar que, en general, el crecimiento de la dorada y la lubina es inferior al de la seriola cuando se emplean fuentes proteicas alternativas, lo que convierte la *S. dumerili* en una especie candidata como alternativa a las especies mediterráneas que más se producen en la actualidad.

2.2. Sustitución del aceite de pescado

La sustitución del aceite de pescado por mezclas de aceites vegetales no afectó de forma significativa al crecimiento, puesto que se cubrieron las necesidades de ácidos grasos esenciales, al igual que ocurre en otras especies mediterráneas, como la dorada (Benedito-Palos et al., 2008; Fountoulaki et al., 2009; Izquierdo et al., 2003; Montero et al., 2008), o la lubina (Izquierdo et al., 2003; Mourente & Bell, 2005).

El siguiente gráfico (Figura 8.2) se resume el crecimiento de los peces de la presente tesis comparándolos con experimentos similares de sustitución lipídica en piensos para otras especies mediterráneas como la dorada (Benedito-Palos et al., 2008; Fountoulaki et al., 2009; Montero et al., 2008) o la lubina (Mourente & Bell, 2005).

La seriola presentó un crecimiento mucho mayor comparado con las otras especies mediterráneas alimentadas con sustituciones lipídicas, a pesar de que, como se ha comentado anteriormente, la sustitución lipídica no parece afectar al crecimiento de ninguna estas especies.

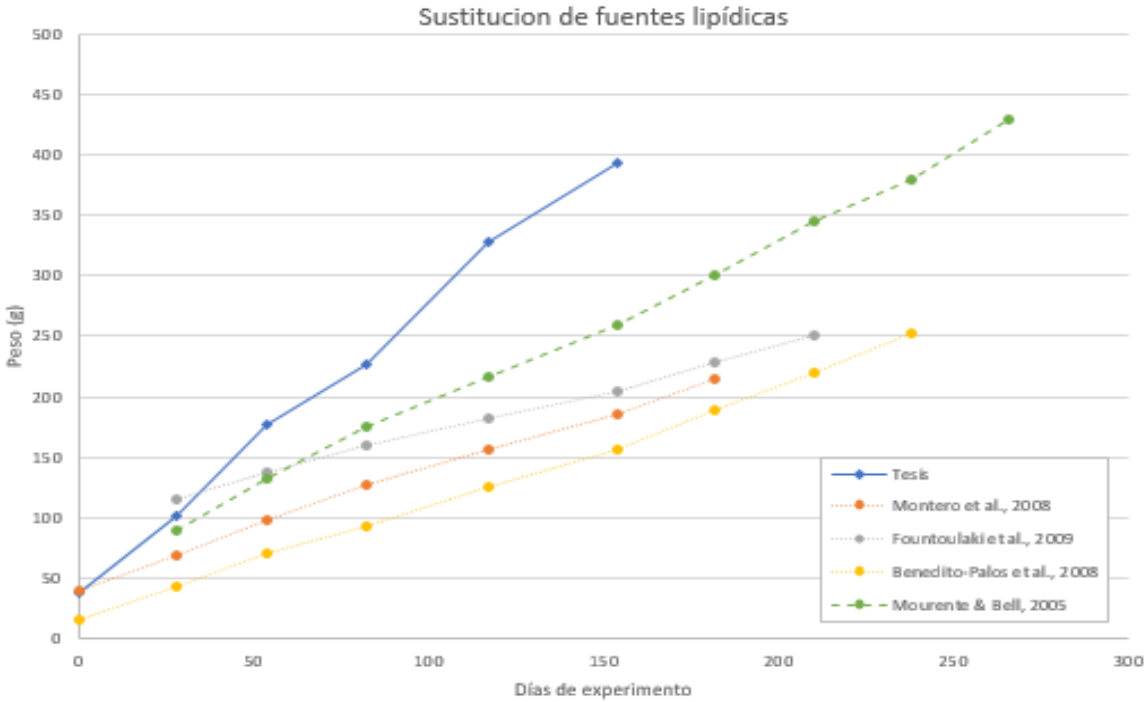

Figura 8.2 Evolución del peso medio de la seriola durante la presente tesis, y dorada y lubina (otros autores), alimentadas con piensos con fuentes lipídicas alternativas. (--- : experimentos realizados en lubina; ····· : experimentos realizados en dorada).

3. Calidad del filete y seguridad alimentaria.

La evaluación del efecto de las distintas dietas en la calidad final del producto al que el consumidor tiene acceso, otro de los objetivos de la presente tesis, ha sido ensayado en las pruebas de sustitución proteica y de sustitución lipídica.

3.1 Sustitución de la harina de pescado

La composición de ácidos grasos de la carne no solo se ve afectada por la sustitución lipídica, también se puede ver afectada por la sustitución de la harina de pescado por fuentes alternativas, debido a que las fuentes vegetales presentan una gran cantidad de ácidos grasos *n*-6, lo que puede afectar al balance *n*-3/*n*-6 del pienso, sin embargo, en este caso está ligeramente compensado con los ácidos grasos procedentes de las fuentes proteicas animales.

La disminución de la harina de pescado en los piensos afecta a los ácidos grasos, especialmente a los *n*-3 PUFA (EPA y DHA). Esta reducción se puede ver en el capítulo 6 de la tesis, especialmente en el pienso con mayor sustitución de harina de pescado, donde el EPA se reduce de 19,65 a 15,07 g/kg y el DHA de 15,33 a 10,52 g/kg. Los peces alimentados con el pienso con mayor sustitución proteica fueron los que menor cantidad de grasa presentaron (un 2% menos en la cantidad de grasa final del filete) afectando, por tanto, a las cantidades finales de ácidos grasos del mismo.

En los resultados obtenidos en la tesis, destacando el caso del EPA y DHA, donde ambos se vieron reducidos notablemente en la carne (los valores entre los peces alimentados con la dieta control y con un 66% de sustitución, se redujeron de 1,4 a 0,51 mg/100g en el caso del EPA y de 3,01 a 1,69 mg/100g en el caso del DHA, respectivamente).

Respecto a los resultados de las pruebas organolépticas, los catadores solo fueron capaces de encontrar diferencias en el aroma de la carne, no en el sabor, por lo que la sustitución proteica no parece afectar a la

calidad organoléptica de la carne de la seriola mediterránea. Esto coincide con estudios previos realizados en otras especies medierráneas, como dorada, lubina o rodaballo, donde la sustitución de harina de pescado por fuentes proteicas alternativas solo afectó al aroma de los filetes (Izquierdo et al., 2003; Regost et al., 2003). Esta ausencia de diferencias no es muy sorprendente, ya que incluso cambios muy marcados en el perfil de ácidos grasos del filete, no han supuesto grandes cambios en la respuesta sensorial por parte de los consumidores en otras especies como la dorada (De Francesco et al., 2007; Izquierdo et al., 2003)

3.2 Sustitución del aceite de pescado

Se calcularon tres índices para evaluar la calidad nutricional de los lípidos del filete de seriola para el consumo humano; el índice aterogénico (IA), el índice trombogénico (IT) y el índice de calidad de los lípidos (FLQ). Los dos primeros se calcularon de la siguiente forma:

$$IA = \frac{C12{:}0 + (4 * C14{:}0) + C16{:}0}{\sum MUFA + n6\ PUFA + n3\ PUFA}$$

$$IT = \frac{C14{:}0 + C16{:}0 + C18{:}0}{(0{,}5 * \sum MUFA + 0{,}5 * n6\ PUFA + 3 * n3\ PUFA) + (\frac{n3PUFA}{n6PUFA})}$$

El índice aterogénico indica el potencial de obstrucción de las arterias, mientras que el índice trombogénico indica el potencial para producir trombos.

En la presente tesis, el índice aterogénico no se vio afectado, mientras que el índice trombogénico aumentó a medida que aumentó la sustitución lipídica (Tabla 8.4).

El índice aterogénico en otras especies como la dorada, se ha visto que se encuentra sobre 0,32 y el índice trombogénico alrededor de 0,21 (Montero et al., 2005; Mourente & Bell, 2005); en lubina el IA se encuentra sobre 0,50 y el IT 0,33 (Grigorakis, 2007) o en el sargo picudo es de 0,51 y 0,24, respectivamente (Rueda et al., 2001). No existe un valor óptimo para estos índices pero, cuanto menores son estos valores, se habla de valores mas favorables en términos de calidad lipídica para el consumo humano.

Los valores de estos índices obtenidos en la tesis (Tabla 8.4) indican que la calidad de la seriola es adecuada para el consumo humano, aunque se ha comprobado que la inclusión gradual de ácidos grasos vegetales tiende a reducir estos valores, pudiendo verse afectada la calidad final del producto.

Tabla 8.4 Índices de calidad lipídica y ratios de ácidos grasos obtenidos en *S. dumerili* en el experimento de fuentes lipídicas (Capítulo 7).

	FO100	**VO50**	**VO100**
Índice de aterogenicidad	0,40	0,39	0,38
Índice de trombogenicidad	0,21^{a}	0,22^{a}	0,25^{b}
Calidad de los lípidos	39,39^{b}	34,88^{b}	28,63^{a}
PUFA/SFA	1,51^{b}	1,48^{ab}	1,41^{a}
n-6/n-3	0,17^{a}	0,24^{b}	0,37^{c}
DHA/EPA	3,02	3,34	3,46
EPA/ARA	5,93	5,56	5,42

Valores con distinto superíndice indican diferencias significativas ($p < 0,05$). n=3.

El índice de calidad de los lípidos indica el porcentaje de relación en que los principales *n*-3 PUFA (EPA y DHA) aparecen en el músculo respecto al total de lípidos; cuanto mayor es este valor, mejor es el valor nutricional del filete. Se ha calculado de la siguiente manera:

$$FLQ = \frac{C20{:}\,5n-3 + C22{:}\,6n-3}{\sum ácidos\ grasos\ totales}$$

En la presente tesis, el valor disminuyó de forma significativa con el aumento de la sustitución del aceite de pescado por aceites vegetales (Tabla 8.4). Se ha observado en dorada que este valor se ha reducido de 23,7 a 13,5 en peces alimentados con piensos sin aceite de pescado (Pérez et al., 2014).

Respecto a los ratios de ácidos grasos, el ratio PUFA/SFA en las dietas humanas se recomienda que sea superior a 0,45 (Wood et al., 2004). En la tabla 8.4 se aprecian los valores obtenidos en los ensayos llevados a cabo en la presente tesis. A pesar de que éste va disminuyendo con el aumento de la sustitución lipídica, sigue siendo muy superior al ratio recomendado (0,45), por lo que se puede concluir que la carne de la seriola es muy adecuada para el consumo humano en este aspecto.

Respecto al ratio *n*-6/*n*-3, se vio que iba en aumento, cuanto mayor era la sustitución lipídica, desde 0,17 hasta 0,37, al igual que ocurre en dorada y lubina, donde aumenta de 0,22 y 0,26 hasta 0,44 y 0,48, respectivamente. Esta proporción *n*-6/*n*-3 tampoco tiene un valor óptimo definido, pero se sabe que cuanto menor sea su valor, más beneficiosos son sus efectos. Como se ve, los valores obtenidos en seriola, son ligeramente inferiores que en dorada y lubina, por tanto la calidad del filete es óptimo para el consumo humano.

En cuanto a los ratios DHA/EPA y EPA/ARA, en la presente tesis la sustitución de fuentes lipídicas no afectó significativamente a este valor y los resultados obtenidos concuerdan con los obtenidos por Rodríguez-Barreto et al., (2012) en seriola, en los que obtuvieron unos valores de 2,4 para el ratio DHA/EPA y 6,4 para EPA/ARA.

4. Análisis económico

Otro de los aspectos importantes a tener en cuenta es la rentabilidad económica a la hora de incluir diferentes materias primas en los piensos.

Se han calculado el índice de conversión económico (ICE), el índice de beneficio económico (IBE) y el índice de beneficio económico estándar

(IBE_{st}) (Jauralde et al., 2013; Martínez-Llorens et al., 2017b) de la siguiente manera:

$$ICE\ (€/kg) = ICA * Precio\ pienso$$

$$IBE\ (€/pez) = \left(P_{fin} * PV\ \right) - \ (ICE * \ Inc.peso)$$

$$IBE_{st}\ (€/pez) = 100 * \frac{Incremento\ peso}{días} * \ (PV - ICE)$$

Donce ICA es la tasa de conversión del alimento, P_{fin} es el peso final del pescado y PV es el precio de venta del pescado en el mercado.

Donde en ICE nos indica el coste necesario para engordar un kilo de pescado y el IBE nos indica el incremento del valor añadido producido por el engorde de un pez, y el IBE_{st} nos indica el incemento del valor añadido de engordar un kilo de pez considerando el gasto realizado por un pienso concreto en un periodo determinado de tiempo.

El precio de los distintos piensos estudiados en el caso de la sustitución lipídica es muy similar (Tabla 8.5.), pero en el caso de la sustitución proteica, el precio se ve reducido a medida que la sustitución aumenta. Se ha visto en experimentos previos que la sustitución por fuentes proteicas alternativas a la harina de pescado, reduce los costes de los piensos debido al elevado precio de la misma en el mercado (Martínez-Llorens et al., 2012; Sánchez Lozano et al., 2007).

En cuanto a los índices económicos obtenidos en la presente tesis (Tabla 8.5.), únicamente se aprecian diferencias significativas en los índices de conversión económicos (ICE) de los experimentos de sustitución proteica, donde se puede observar que, en ambos casos, es significativamente más rentable la sustitución por fuentes proteicas alternativas, ya que como se ha comentado previamente, el valor de la harina de pollo y de krill desengrasado es muy inferior al de la harina de pescado.

Tabla 8.5 Índices económicos obtenidos en los experimentos de la tesis.

		Precio Pienso (€/kg)	ICE	IBE	IBE_{st}
Sust. proteica I (Cap. 5)	**FM100**	1,27	2,22[a]	4,31	2,46
	FM66	1,05	1,77[b]	4,46	2,57
	FM33	0,83	1,46[c]	4,08	2,32
	FM0	0,65	1,17[d]	3,91	2,23
	P-valor		0,0002	0,5297	0,5235
Sust. Proteica II (Cap. 7)	**FM100**	1,27	1,96[a]	10,49	4,36
	FM66	1,05	1,53[b]	10,53	4,39
	FM33	0,83	1,38[c]	9,55	3,03
	P-valor		0,0003	0,3602	0,3585
Sust. Lipídica I (Cap. 6)	**FM100**	1,27	2,21	4,31	2,46
	VO50	1,22	2,11	4,37	2,52
	VO100	1,17	2,01	4,23	2,41
	P-valor		0,1998	0,7920	0,6896

ICE: índice de conversión económico; IBE: índice de beneficio económico. IBEst: IBE estándar. n=3, diferentes superíndices en cada columna indican diferencias significativas ($p < 0.05$) test de Newman-Keuls.

Mientras que el ICE depende demasiado del precio de la dieta, el índice de beneficio económico (IBE) parece más adecuado a la hora de comparar la rentabilidad económica, ya que tiene en cuenta un mayor número de parámetros. Como se puede observar en los experimentos llevados a cabo, los valores entre las diferentes dietas son bastante similares.

Si se comparan los valores del IBE con otras especies mediterráneas, como dorada (Martínez-Llorens et al., 2007; Moutinho et al., 2016; Sánchez Lozano et al., 2007), que presentaron valores entre 1,28 y 1,43 €/kg en los mejores piensos, respectivamente; o lubina, con valores de 1,8 €/kg (Adaklı & Taşbozan, 2015) o sargo, con valores de 1,5 €/kg (Hernández et al., 2007) vemos que en todos los casos son muy inferiores a los datos obtenidos en seriola de la presente tesis, lo que nos indica que la seriola es una especie más rentable desde el punto de vista económico.

5. Criterios de sostenibilidad, Índice FIFO

Otro de los parámetros que se han tenido en cuenta es el índice FIFO (del inglés "*fish in: fish out ratio*") o ratio de pescado requerido : pescado obtenido, utilizado para evaluar la sostenibilidad de la acuicultura en relación al pescado salvaje que es utilizado en la fabricación del alimento (Tacon & Metian, 2008).

Así, se usa como referencia de progreso del sector en cuanto a su rendimiento medioambiental, y se ha convertido en la medida principal utilizada para garantizar que la acuicultura no afecta negativamente a las poblaciones de peces silvestres (Kok et al., 2020).

La sustitución de la harina y el aceite de pescado son un punto de partida importante en la reducción de este ratio, que se calcula de la siguiente manera:

$$\text{FIFO} = \left[\frac{\%\ harina\ pienso + \%\ aceite\ pienso}{RH + RA}\right] * ICA$$

Donde RH es el rendimiento de la harina de pescado (24%), RA el rendimiento del aceite de pescado (5%), que se obtienen aproximando que de cada tonelada de pescado silvestre, se obtienen 240kg de harina y 50kg de aceite de pescado (Tacon & Metian, 2008) e ICA: índice de conversión del alimento (pienso ingerido/incremento de biomasa).

Los índices FIFO se han reducido notablemente en los últimos años. Actualmente el FIFO global de la acuicultura se encuentra en niveles inferiores a 1, gracias a la reducción que la harina y el aceite de pescado han tenido en los piensos para estos peces a lo largo del tiempo.

Estos resultados son muy positivos. La industria de la harina de pescado produce un volumen de proteína total para la humanidad mayor del que se suministraría simplemente por el consumo directo del pescado utilizado como materia prima en esta.

Los índice FIFO obtenidos en la presente tesis se muestran en la tabla 8.6.

Tabla 8.6 Indices FIFO obtenidos con las dietas utilizadas en la presente tesis doctoral.

	Pienso	Harina de pescado (g/kg)	Aceite de pescado (g/kg)	Índice FIFO
Fuentes Proteicas I (Cap. 5)	FM100	525	90	3,75
	FM66	350	92	2,56
	FM33	175	88	1,57
	FM0	0	95	0,58
Fuentes Proteicas II (Cap. 7)	FM100	525	90	3,28
	FM66	350	92	2,21
	FM33	175	88	1,49
Fuentes Lipídicas (Cap. 6)	FM100	525	90	3,75
	VO50	525	45	3,43
	VO100	525	0	3,11

Los índices FIFO obtenidos no presentan unos buenos valores respecto a otras especies de acuicultura, pero sí si se comparan con otros experimentos realizados previamente en *Seriola dumerili* se puede apreciar como en Tomás et al. (2005) obtuvieron índices FIFO entre 5,43 y 9,14 en función del nivel de sustitución de la harina de pescado por torta de soja, pero sigue estando lejos de los valores ideales.

Dawood et al. (2015), en piensos con sustitución proteica en seriola, obtuvieron valores similares entre todas sus dietas (entre 1,41 – 2,41).

En otras especies mediterráneas, como la dorada, los piensos comerciales formulados con 400 g HP/kg y 150 g AP/kg presentan un índice FIFO de 4,6 (Kaushik & Troell, 2010), mientras que en experimentos con sustituciones de harinas y aceites por otras fuentes se ha reducido hasta 1,7 (Benedito-Palos, 2010). La elevada necesidad de suminstrar proteínas de alta calidad para la alimentación de la *Seriola dumerili* es algo que se deberá abordar en mayor profundidad en experimentos futuros, probando otro tipo de materias primas

alternativas, si se quiere prescidir totalmente de las harinas y aceites de pescado.

Capítulo 9
CONCLUSIONES

De los resultados obtenidos en la presente tesis doctoral se puede concluir que:

- ✓ Las fuentes proteicas animales son las más adecuadas para la sustitución de la harina de pescado en piensos de seriola mediterránea debido a que las fuentes proteicas vegetales presentaron deficiencias nutricionales y baja digestibilidad, por lo que deben combinarse con otras materias primas proteicas o sumplementos nutricionales.

- ✓ La sustitución de un 66% de la harina de pescado por fuentes proteicas animales y vegetales no afectó ni al crecimiento ni a la supervivencia de los peces, siendo la dieta con mayor eficiencia nutritiva y económica.

- ✓ La sustitución parcial de la harina de pescado durante un periodo superior a 10 meses tuvo un efecto negativo en el crecimiento con una sustitución de la harina de pescado del 66%, pero no afectó a la composición nutricional del filete. El nivel de metales pesados, excepto el arsénico, estuvo por debajo de los niveles marcados por la UE, los ácidos grasos se ven reducidos a medida que aumenta la sustitución, y los parámetros fisicoquímicos y organolépticos sí se vieron afectados por la sustitución proteica, pero no parecen tener un efecto en la apreciación que tiene el consumidor sobre el producto final.

- ✓ La utilización de aceite vegetal (PO:LO 4:1) puede sustituir completamente el aceite de pescado en juveniles de *S. dumerili* sin afectar al crecimiento ni a la utilización del alimento.

- ✓ El perfil de ácidos grasos esenciales en los piensos con una sustitución parcial y total del aceite de pescado cubre las necesidades de los juveniles de la *Seriola dumerili* sin afectar a la supervivencia de los mismos, presentando unas características nutricionales satisfactorias para el consumo humano.

La *Seriola dumerili* se ha posicionado como una especie idónea para la acuicultura por su rápido crecimiento, su alto precio de mercado, su calidad de la carne y su adaptación a la cautividad; sin embargo, a pesar de que la sustitución total del aceite de pescado por fuentes lipídicas alternativas ha reflejado unos buenos resultados, tanto en el crecimiento como en la calidad del producto final, la sustitución total de la harina de pescado por fuentes proteicas produjo un bajo crecimiento y una elevada mortalidad, lo que hace necesario evaluar nuevos ingredientes proteicos o aditivos que mejoren la inmunidad de los peces y aumenten la efectividad de los piensos sin harinas de pescado para el engorde de esta especie a nivel comercial.

Capítulo 10
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