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

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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 fishmeal substitution, and FM66 and FM33 diets with a fishmeal replacement of 330 g/kg and 660 g/kg, respectively. At the end of experiment, fish fed the FM66 diet showed the no differences in growth, nutritional parameters and fatty acid composition. 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 diets, affects *Seriola dumerili* growth; despite this the quality of the fillet was not affected even with a 66 % of substitution.

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

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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 et al. (1997), *Senegalese sole,* Valente et al. (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^{-1} 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^{-1}. Water salinity was 31.5 ± 4.1 g L^{-1}, pH 7.3 ± 0.4, NO_3-25−150 mg L^{-1}, NO_2-0.05–0.5 mg $L^{-1}$and the ammonium value was undetectable. All values were measured three times a week. The photoperiod was natural throughout the experimental period (16 L/8D in summer and 12 L/12D in winter) and all tanks had similar lighting conditions.

A group of 90 fish (average weigh 530 g), 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 °C to determine proximate composition, fatty acids, heavy metals and sensory analysis.

Three isolipidic (140 g kg^{-1} of crude lipid) and isoenergetic diets (24 MJ kg^{-1} of gross energy) were formulated (Table 1) with the same digestible protein level (50 % DP), and from 530 to 604 g $kg^{-1}$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

Table 1
Ingredients and chemical and heavy metals composition of the experimental diets.

	FM100	FM66	FM33
Ingredients (g kg^{-1} 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
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^{-1})	23.8	24.1	23.7
DP (g Kg^{-1}) [10]	497	504	497
DE (MJ Kg^{-1}) [10]	20.3	19.2	18.1
DP/DE ratio (g MJ^{-1})	24.5	26.2	27.5
Heavy metals (mg kg^{-1} DM)			
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^{-1} 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^{-1}): 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^{-1}; calcipherol. 500 IU kg^{-1}; 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.
[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).

protein source (525 g kg^{-1}), 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^{-1}, 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^{-1} of dry matter. The composition of the experimental diets and their proximate values are shown in Table 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 °C 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 (9.00 h and 17.00 h), 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).

All fish were individually weighed at intervals of 30 days approximately. Prior to weighing, the fish were anaesthetized with 30 mg L^{-1} 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 h 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 °C to constant weight), ash (incinerated at 550 °C to constant weight), ether extract was determined using an Ankom XT10 Extraction System (NY, USA) according to AOCS (2005); nitrogen content (crude protein × 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 et al. (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 × 0.25 mm x0.2 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 °C held for five min and increased to 240 at 4 °C/min and finally maintained at that temperature for 30 min. Both detector and injector temperatures were set at 260 °C. 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 2.

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 1.

Table 2
Fatty acid composition of the experimental diets (g kg^{-1} DM).

Fatty acids	FM100	FM66	FM33
SFA			
C 13:0	3.57	3.32	4.04
C 14:0	7.34	7.22	6.78
C 15:0	0.48	0.45	0.41
C16:0	24.2	24.7	26.3
C 17:0	0.44	0.42	0.43
C18:0	4.44	5.20	6.71
C 20:0	0.21	0.38	0.28
C 22:0	0.20	0.22	0.14
MUFA			
C 16:1	8.50	7.87	7.00
C 17:1	0.13	0.14	0.17
C 18:1n7	3.60	3.71	3.92
C 18:1n9c	13.14	16.29	20.87
C 20:1	0.71	0.65	0.64
C 24:1	0.42	0.31	0.26
PUFA			
C 18:2n6c (LA)	8.41	9.85	11.21
C 18:3n6	0.23	0.21	0.22
C 18:3n3 (LNA)	1.21	1.39	1.66
C 20:2	2.48	2.83	2.90
C 20:3n6	0.17	0.15	0.13
C 20:4n6 (ARA)	1.01	0.87	0.74
C 20:5n3 (EPA)	19.65	17.74	15.07
C 22:2	0.74	0.67	0.59
C 22:5n3	2.07	1.70	1.31
C 22:6n3 (DHA)	15.33	12.85	10.52
n3 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; n3 HUFA: Highly unsaturated fatty acids; LA: Linoleic acid; LNA: Linolenic acid; ARA: Arachidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

Six samples of fillets of each treatment were lyophilized for heavy metals analysis. 0.5 g of lyophilized sample was digested with 5 mL HNO_3Suprapur® in Teflon vials to decompose the samples at 100 °C for 3 h. 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 °C. Prior to sampling, fish were thawed in a refrigerator at 4 °C 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* (Robertson, 1976) of 15fish 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 10° (UNE 7203). CIE Lab coordinates L* (lightness), a* (redness), and b* (yellowness) were used to calculate colour difference, ΔE (compared to control sample, FM100) (Eq. (1)) and chroma (Eq. (2)). Six measures were performed at different points of each sample.

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

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

The moisture content was determined by oven drying at 105 °C for 20−24 h or until constant weight (Association of Official Analytical Chemists (AOAC, 1995). 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 s 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 50 × 50 × 15 mm (wide×long×thick). Seven textural parameters were determined from each curve: hardness (N), adhesiveness (N.s), springiness, cohesiveness, gumminess, chewiness and resilience (Bourne, 1978). 15samples 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 (fresh fish, seaweed or sea) (REGULATION(EC) 2406/96) (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 °C (Codex Alimentarius, 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 and 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 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).

3.2. Biometrics, body composition and nutrient retentions

Data on biometric parameters and body composition of fish fed different diets are shown in Table 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 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

Table 3
Overall 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^{-1}) [2]	0.56[a]	0.56[a]	0.41[b]	0.02
FI (g 100 g $fish^{-1}$ day^{-1}) [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

Means with different superscripts indicate significantly differences ($p < 0.05$). SEM: standard error of the mean (n=3). Newman−Keuls test.

[1] Initial weight was considered as covariable for final weight and specific growth rate.

[2] Specific growth rate (SGR) = 100 × ln (final weight/initial weight)/days.

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

[4] Feed conversion ratio (FCR) = feed consumption (g) / weight gain (g).

[5] Protein efficiency ratio (PER) = weight gain (g) / crude protein intake (g).

Table 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^{-1})	6.3	6.3	6.0	0.2
Biometric indices (n = 15)				
CF (g cm^{3-1}) [1]	1.55[a]	1.58[a]	1.38[b]	0.06
VSI (%) [2]	4.84	5.41	4.83	0.42
HSI (%) [3]	0.98	0.92	1.01	0.05
VFI (%) [4]	0.09	0.13	0.02	0.05
MI [5]	46.6	46.6	47.2	1.1
PNEP [6]	50.3	49.6	50.5	0.8
DP (%) [7]	65.2	64.9	64.5	1.3
Efficiency retention (n = 3)				
Crude protein efficiency[8]	31.7	32.5	23.8	3.0
Crude lipid efficiency[9]	36.4[b]	53.3[a]	7.1[c]	7.8
Gross energy efficiency[10]	21.0[a]	17.2[b]	16.5[b]	2.8

Means in the same row with different superscript letters are significantly different (p<0.05). SEM: standard error of the mean. Newman−Keuls test.

[1] Condition factor (CF) (g cm^{3-1}) = (total fish weight (g) / length^3 (cm)) × 100.

[2] Viscerosomatic index (VSI) (%) = (visceral weight (g) / total fish weight, (g)) × 100.

[3] Hepatosomatic index (HIS) (%) = (liver weight (g) / total fish weight (g)) × 100.

[4] Visceral fat index (VFI) (%) = (visceral fat (g) / total fish weight (g)) × 100.

[5] Meat index, MI = (fillet weight (g) / total fish weight (g)) × 100.

[6] Percentage of non-edible portion (PNEP) = ((head + fins + rachis + visceral weight, g) / total fish weight (g) × 100.

[7] Dressout percentage (DP) (%) = ((total fish weight- head-viscera weight (g) / total fish weight (g)) × 100.

[8] Crude Protein Efficiency (%) = (fish protein gain (g) × 100) / protein intake (g).

[9] Crude lipid Efficiency (%) = (fish lipid gain (g) × 100) / lipid intake (g).

[10] Gross Energy Efficiency (%) = (fish energy gain (kJ) × 100) / energy intake (kJ).

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).

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 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.

3.4. Heavy metals

Copper increased its values in the different diets with a wide difference between them (Table 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 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^{-1} DM) of mercury and cadmium were obtained. The concentration of zinc in muscle varied from 25.2–30.5 mg kg^{-1} DM, without differences between diets ($p < 0.05$).

Table 5
Fatty acid composition (mg 100 g^{-1} 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
C16:0	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
C18:1n-7	0.80[a]	0.91[a]	0.36[b]	0.14
C18:1n9c	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
C18:2n6c (LA)[4]	3.22[a]	2.77[a]	1.44[b]	0.28
C1 8:3n3 (LNA)[5]	0.40[a]	0.31[ab]	0.17[b]	0.11
C20:4n6 (ARA)[6]	0.19[ab]	0.23[a]	0.12[b]	0.02
C20:5n3 (EPA)[7]	1.40[a]	1.04[a]	0.51[b]	0.12
C22:5n-3	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
C22:6n3 (DHA)[8]	3.01[ab]	3.71[a]	1.69[b]	0.14
n-3 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 (n= 3) in the same row with different superscript letters are significantly different (p<0.05). Newman−Keuls test.
Crude lipid muscle (% of dry matter): 25.5 % FM100; 22.6 % FM66 and 12.2 % FM33.

[1] SFA: Saturated fatty acids.
[2] MUFA: Mono unsaturated fatty acids.
[3] PUFA: Poly unsaturated fatty acids.
[4] LA: Linoleic acid.
[5] LNA: Linolenic acid.
[6] ARA: Arachidonic acid.
[7] EPA: Eicosapentaenoic acid.
[8] DHA: Docosahexaenoic acid.
[9] n-3 HUFA: Highly unsaturated fatty acids.

Table 6
Heavy metals composition of Mediterranean yellowtail fillets (mg 1000 g^1DM) fed the experimental diets.

	FM100	FM66	FM33	SEM
Arsenic	1.40[a]	1.38[a]	0.81[b]	0.08
Cadmium	0.04	0.00	0.01	0.01
Cooper	1.34	1.42	2.60	0.35
Mercury	0.13	0.11	0.12	0.009
Zinc	25.2	26.6	30.5	1.7

Means in the same row with different superscript letters are significantly different (p<0.05). SEM: pooled standard error of the mean (n=6). Newman−Keuls test.

3.5. Sensory analysis: Physicochemical and mechanical analysis

Table 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 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 ($p < 0.05$).

3.6. Sensory analysis: organoleptic evaluation

In the sensory analysis (Fig. 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.

4. Discussion

4.1. 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 et 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 and 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 and 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 (Sitjà-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-Ariño 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).

Ina 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 345 g above 391 and 385 g) (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-Vidal 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 and Tope, 2017), or including low amount alternative marine ingredients to recover the gut mucosa functionality (Estruch et al., 2020).

Table 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^{a}	-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
Chromaticity (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 (n= 15) in the same row with different superscript letters are significantly different (p <0.05). SEM: pooled standard error of the mean. Newman-Keuls test.

4.2. 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.

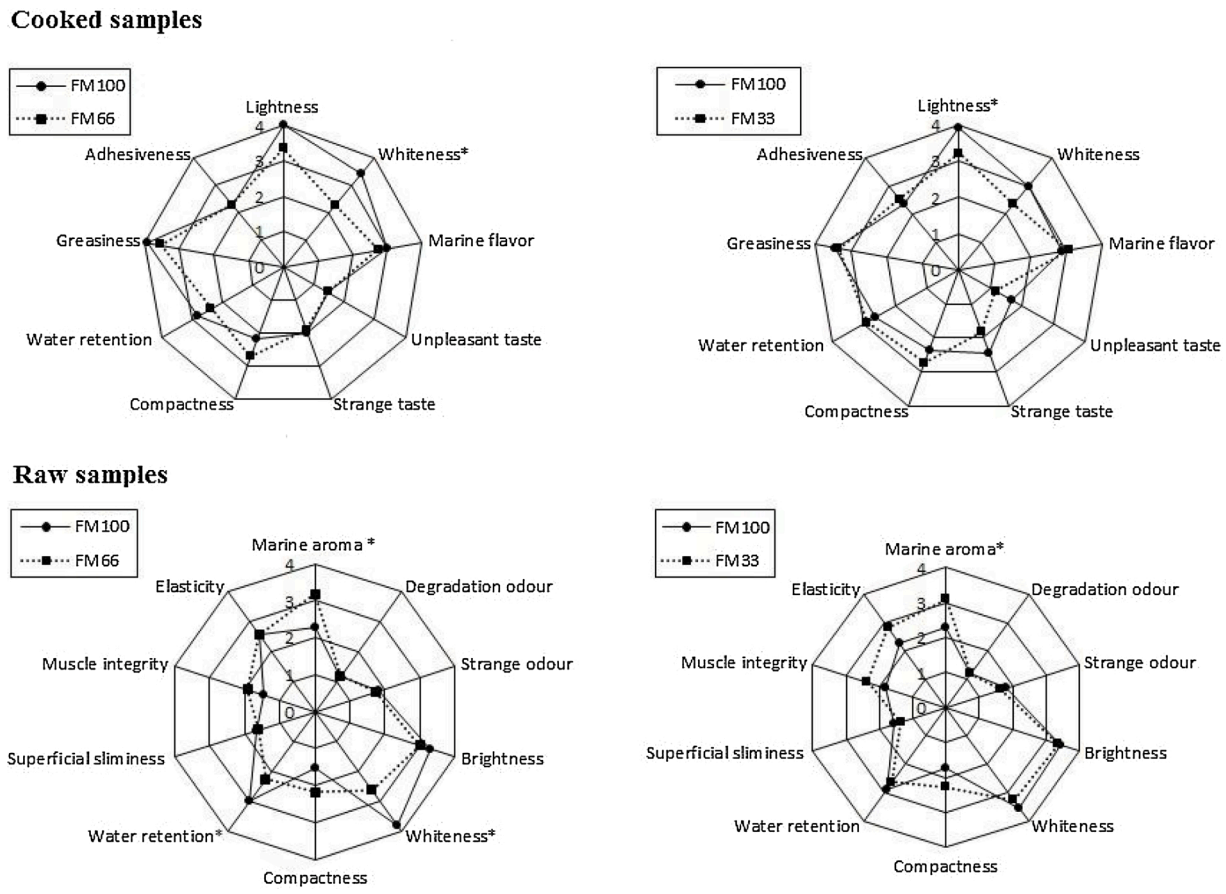

Fig. 1. Organoleptic test of *S. dumerili* samples of muscle. Comparison between FM100-FM66 and FM100-FM33 in raw and cooked samples. Significant differences ($P < 0.05$) are shown with (*) in the graphics. Newman-Keuls test.

4.3. 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 and 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 (Edmonds and 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^{-1} 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).

4.4. 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.

Data availability statement

Research data are not shared.

CRediT authorship contribution statement

R. Monge-Ortiz: Methodology, Investigation, Writing - review & editing. **S. Martínez-Llorens:** Methodology, Investigation, Supervision. **M.J. Lemos-Neto:** Investigation, Methodology. **S.L. Falcó-Giaccaglia:** Methodology, Investigation. **M.J. Pagán:** Methodology, Investigation. **S. Godoy-Olmos:** Metodology. **M. Jover-Cerdá:** Supervision. **A. Tomás-Vidal:** Methodology, Investigation, Supervision, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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