Aquaculture Reports 25 (2022) 101262

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Modulation of dietary protein to lipid ratios for gilthead seabream on-growing during summer temperature conditions

Cláudia Aragão [a,b,*], Miguel Cabano [a], Rita Colen [a], Rita Teodósio [a,b], Enric Gisbert [c], Jorge Dias [d], Sofia Engrola [a]

[a] Centre of Marine Sciences (CCMAR), 8005-139 Faro, Portugal
[b] Universidade do Algarve, 8005-139 Faro, Portugal
[c] IRTA, Aquaculture Program, 43450 Sant Carles de la Ràpita, Tarragona, Spain
[d] SPAROS Lda., 8700-221 Olhão, Portugal

ARTICLE INFO

Keywords:
Mediterranean aquaculture
Sparus aurata
Nutrient digestibility
Lipid retention
Nitrogen losses

ABSTRACT

Gilthead seabream (*Sparus aurata*) tend to increase fat deposition during summer farming conditions in the Mediterranean, which may negatively affect productive performance and consumers' quality perception of the final product. Therefore, this study evaluated the impacts of protein to lipid ratios in low fishmeal/fish oil diets on growth performance, body composition, feed conversion and nutrient utilization of seabream on-grown during summer temperature conditions. The experimental diets contained low levels of fishmeal, fish oil, and crude protein (39%), differing in crude lipid content: 16% (MF diet) or 12% (LF diet). A growth trial was performed with seabream (initial weight: 100 ± 7 g) from August to October (water temperature: 23.1 ± 2.2 °C). A digestibility trial was also performed (at 23 °C). Key performance indicators, whole-body composition and activities of digestive enzymes were evaluated at the end of the experiment (64 days). Low dietary lipid levels negatively affected lipid, energy, and amino acid digestibility, and as a result, fish fed the LF diet presented higher nitrogen faecal losses. Still, the decrease in nutrient digestibility was not related to dietary effects on the digestive enzyme activities. The experimental diets did not compromise the activity of pancreatic, gastric, and intestinal digestive enzymes nor feed utilization, but a slight growth impairment was observed in fish fed the LF diet, probably due to the lower amino acid and lipid digestibility. However, a potential benefit of this dietary treatment towards reducing fat accumulation in seabream during summer was observed. Nevertheless, the environmental impact of the nitrogen losses during seabream on-growing should be considered when estimating the sustainability of the production. This study demonstrated that the optimisation of diet formulations should account for the environmental conditions, especially in Mediterranean aquaculture, so the economic and environmental impacts may be correctly evaluated towards a more sustainable fish production.

1. Introduction

Scientific data indicate that fish consumption brings health benefits, and this has been one of the factors that have driven a steady increase in fish consumption over the years (Tacon et al., 2020). In addition, the growth of aquaculture has been fuelled by the expansion in global trade, declines in the availability of wild fish, competitive product pricing, rising incomes, and urbanization, all of which contribute to increasing per capita consumption of seafood worldwide (Naylor et al., 2021). Fish consumers demand product quality and are concerned with the environmental impacts of the aquaculture industry (Gartzia et al., 2018). The flesh composition and nutritional value of the fish are some of the characteristics that may define fish quality and the consequent consumer acceptance. Both characteristics are affected by intrinsic fish factors, such as species, age, and sex, but also by extrinsic factors, like water temperature and feed composition (Shearer, 1994; Grigorakis, 2007). Farmed gilthead seabream (*Sparus aurata*) tends to accumulate a significantly higher amount of peritoneal and perivisceral fat than their wild counterparts (Grigorakis et al., 2002). Furthermore, body composition of gilthead seabream changes seasonally, especially in lipid content, with minimum values observed in late spring and maximum ones in late summer (Grigorakis et al., 2002; Pleadin et al., 2015). Water

* Correspondence to: Centre of Marine Sciences of Algarve (CCMAR), Universidade do Algarve, Campus de Gambelas, building 7, 8005-139 Faro, Portugal.
E-mail address: caragao@ualg.pt (C. Aragão).

https://doi.org/10.1016/j.aqrep.2022.101262
Received 23 November 2021; Received in revised form 27 June 2022; Accepted 12 July 2022
Available online 14 July 2022

temperature affects feed intake, digestion, metabolism, and growth of fish since they are poikilothermic animals. An increase in fat content during the summer is indicative of the preparation of fish bodies for the winter, after a long period of intense feeding (Pleadin et al., 2015). However, fat deposits are usually not well perceived by the consumers and affect fish shelf-life, especially in those species that are commercialized as whole fish (Grigorakis, 2007; Maestre et al., 2011).

Growth optimisation is intrinsically connected with an increase in protein retention and an efficient use of dietary protein is also of utmost importance to minimise the environmental impacts of the aquaculture industry. Protein utilisation is dependent on several factors, including ingredient digestibility, dietary amino acid profile, and protein and lipid contents (Halver and Hardy, 2002). An increase in dietary lipid content has been shown to have a protein-sparing effect in some fish species, especially in salmonids (e.g., Karalazos et al., 2011; Yigit et al., 2002), resulting in an improvement in growth performance. However, this effect is not so apparent in other fish species. In gilthead seabream, some studies suggested a protein-sparing effect of lipids up to some extent (Company et al., 1999; de la Serrana et al., 2013; Mongile et al., 2014), but mostly without impacts on growth performance (Bonaldo et al., 2010; Mongile et al., 2014; Santinha et al., 1999; Velázquez et al., 2006). Furthermore, while some authors reported an increase in lipid accumulation with increasing dietary lipid levels (Company et al., 1999; de la Serrana et al., 2013; Santinha et al., 1999), others related no effects on lipid deposition (Bonaldo et al., 2010; Mongile et al., 2014; Velázquez et al., 2006). In fact, dietary lipid levels have been shown to influence digestive enzyme activities and nutrient digestibility and absorption (Chang et al., 2018; Fountoulaki et al., 2005; García-Meilán et al., 2016; Li et al., 2012), which ultimately may be reflected on nutrient metabolism and retention. These effects are dependent not only on the dietary protein and lipid contents but are also species- and stage-specific. This may explain the differences among studies and reinforce the need for specific experiments focusing on the target fish species, fish weight, and farming conditions.

A previous study has shown that feeding seabream with a diet containing low fishmeal and protein levels and a mix of feed additives during winter improved fish performance and minimised nitrogen losses to the environment (Teodósio et al., 2021). Growth performance of gilthead seabream under farming conditions is optimal at 24–26 °C (Hernández et al., 2003), which reflects the average summer temperature in the Mediterranean Sea (seatemperature.org, 2021). However, as reviewed above, farmed gilthead seabream increases fat deposition during this period, which may negatively affect productive performance and consumers' quality perception. Therefore, this study aimed at evaluating the impacts of protein to lipid ratios in low protein and low fishmeal/fish oil diets on growth performance, body composition, feed conversion and nutrient utilization of gilthead seabream on-grown during summer temperature conditions.

2. Material and methods

2.1. Diets and fish

Two experimental diets were formulated (Table 1) with the same crude protein (39% CP) but differing in crude lipid (CL) contents: 16% (MF diet) and 12% (LF diet). Both diets had low levels of fishmeal inclusion (13%), whereas a mixture of fish and rapeseed oils (at a fixed ratio of 30:70) was used as the main lipid source. Diets were formulated to fulfil the known nutritional requirements (indispensable amino acids and phosphorus) of juvenile gilthead seabream. Proximate composition of experimental diets is presented in Table 1.

All diets were manufactured at SPAROS Lda. (Olhão, Portugal) by extrusion (pellet size 4.0 mm), using a pilot-scale twin-screw extruder (CLEXTRAL BC45, Clextral, France) with a screw diameter of 55.5 mm and temperature ranging from 105 to 110 ºC. Upon extrusion, feeds were dried in a vibrating fluid bed dryer (model DR100; TGC Extrusion, France). Following drying, pellets were allowed to cool at room temperature, and subsequently the oil fraction was added under vacuum coating conditions in a Pegasus vacuum mixer (PG-10VCLAB, DINNISEN, The Netherlands). During the trial, all experimental diets were stored in a cool and aerated room.

Gilthead seabream were obtained from Aqualvor-Actividades em Aquacultura Lda. (Odiáxere, Portugal) and transported to the

Table 1
Formulation and proximate composition of the experimental diets.

Ingredients (%)	MF	LF
Fishmeal[a]	13.00	13.00
Haemoglobin[b]	2.00	2.00
Poultry meal[c]	5.00	5.00
Soy protein concentrate[d]	8.00	8.00
Wheat gluten[e]	4.00	4.00
Corn gluten[f]	11.00	11.00
Soybean meal[g]	16.00	16.50
Rapeseed meal[h]	5.00	5.00
Wheat meal[i]	12.74	14.24
Whole peas[j]	4.00	4.00
Cellulose[k]	0.00	2.00
Fish oil[l]	4.50	3.30
Rapeseed oil[m]	10.50	7.70
Vitamin & mineral premix[n]	1.00	1.00
Antioxidant powder[o]	0.20	0.20
Sodium propionate[p]	0.10	0.10
Monoammonium phosphate[q]	1.35	1.35
L-Lysine[r]	0.60	0.60
L-Threonine[s]	0.08	0.08
L-Tryptophan[t]	0.05	0.05
DL-Methionine[u]	0.38	0.38
L-Taurine[v]	0.50	0.50
Proximate composition (% as fed)*		
Dry matter	91.3	92.1
Ash	7.2	7.2
Crude protein	39.0	39.4
Crude lipid	15.9	12.4
Total phosphorus	0.8	0.8
Gross energy (MJ kg^{-1})	19.9	19.3

[a] Fish meal NORVIK 70: 70.3% crude protein (CP), 5.8% crude lipids (CL); Sopropêche, France.
[b] Haemoglobin powder 92 P: 91% CP, 1.2% CL; SONAC B.V., The Netherlands.
[c] Poultry meal 65: 67% CP, 12% CL; SAVINOR UTS, Portugal.
[d] Soycomil P: 63% CP, 8% CL; ADM, The Netherlands.
[e] VITAL: 80% CP, 7.5% CL; Roquette Frères, France.
[f] Corn gluten meal: 61% CP, 6% CL; COPAM, Portugal.
[g] Solvent extracted dehulled soybean meal: 47% CP, 2.6% CL; CARGILL, Spain.
[h] Defatted rapeseed meal: 34% CP, 2% CL; Premix Lda, Portugal.
[i] Wheat meal: 10% CP, 1.2% CL; Casa Lanchinha, Portugal.
[j] Yellow peas: 19.6% CP, 2.2% CL; Ribeiro e Sousa Lda., Portugal.
[k] DISPROQUíMICA, Portugal.
[l] Sopropêche, France.
[m] J.C. Coimbra Lda., Portugal.
[n] INVIVONSA Portugal AS, Portugal: Vitamins (IU or mg kg^{-1} diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 500 mg; inositol, 500 mg; biotin, 3 mg; calcium pantothenate, 100 mg; choline chloride, 1000 mg; betaine, 500 mg. Minerals (g or mg kg^{-1} diet): copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate, 7.5 mg; sodium chloride, 400 mg; excipient wheat middling's.
[o] Paramega PX, KEMIN EUROPE NV, Belgium.
[p] Disproquímica, Portugal.
[q] Windmill Aquaphos: 26% P; ALIPHOS ROTTERDAM B.V, The Netherlands.
[r] Biolys: L-lysine sulphate, 54.6% lysine; EVONIK Operations GmbH, Germany.
[s] L-Threonine: 98%; EVONIK Operations GmbH, Germany.
[t] L-Tryptophan: 98%; EVONIK Operations GmbH, Germany.
[u] DL-Methionine: 99%; EVONIK Operations GmbH, Germany.
[v] L-Taurine: 98%; ORFFA, The Netherlands.
*All values are reported as the mean of duplicate analysis.

Experimental Research Station of the Centre of Marine Sciences (CCMAR, Faro, Portugal). Fish were adapted to the new conditions in a flow-through system with aeration and were fed to apparent satiety with a commercial diet (Standard 4 Orange, Sorgal, Portugal; 43% CP, 17% CL).

Experiments were directed by trained scientists (following the Federation of European Laboratory Animal Science Associations – FELASA category C recommendations) and were conducted according to the European (Directive 2010/63/EU of European Parliament and the Council of the European Union) and Portuguese (Decreto-Lei nº 113/2013 de 7 de Agosto) legislation on the protection of animals used for scientific purposes.

2.2. Diet digestibility

Six homogenous groups of nine seabream each (average weight: 113 ± 1 g) were stocked in cylinder-conical fibreglass tanks of 100 L, in which the outlet water run through a recipient adapted to serve as a faeces settling decantation system. The apparent digestibility coefficients (ADC) of the dietary components were determined by the indirect method, using 1% chromic oxide as an inert tracer. Fish were fed by hand with the experimental diets containing chromic oxide, once a day to apparent satiety. After an adaptation period of six days, faeces collection started. Each day, after feeding, the tanks were thoroughly cleaned to remove any uneaten feed, and the fish were maintained in the tanks with clean seawater (± 23 ºC) and aeration. Faecal samples were collected eight to nine hours after feeding. Pooled samples from the same tank were frozen at − 20 ºC until analysis.

The ADC of the dietary nutrients and energy were calculated as follows (Maynard et al., 1979):

$$\text{ADC} \quad (\%) = 100 \times \left[1 - \frac{\text{dietary } Cr_2O_3 \quad \text{level}}{\text{faecal } Cr_2O_3 \quad \text{level}}\right] \times \left[\frac{\text{faecal nutrient or energy } \textit{level}}{\text{dietary nutrient or energyl evel}}\right]$$

The ADC of dry matter was calculated as:

$$\text{ADC} \quad (\%) = 100 \times \left[1 - \frac{\text{dietary } Cr_2O_3 \quad \text{level}}{\text{faecal } Cr_2O_3 \quad \text{level}}\right]$$

2.3. Growth trial

In a parallel experiment, six homogenous groups of 40 seabream each (initial average weight: 100 ± 7 g) were stocked in cylinder fibreglass tanks of 500 L at an initial density of 8.0 kg m^{-3}. At this moment, 30 fish from the initial stock were sampled, measured, and weighed individually, and five of these fish were pooled and stored at − 20 ºC for analysis of whole-body composition. Rearing tanks were supplied with flow-through, gravel-filtered, aerated seawater and subjected to natural photoperiod changes through summer conditions (August-October; 37º0′22.496″, 7º58′2.809″). Environmental parameters were monitored daily (temperature: mean 23.1 ± 2.2 ºC, with a maximum value of 26.1 ºC and a minimum value of 17.1 °C during the experiment; salinity: 35.4 ± 0.3‰; oxygen content in water: > 80% saturation). Daily water temperature data is presented in Supplementary Fig. 1. Each experimental diet was assigned to triplicate tanks and tested for 64 days. Fish were fed by hand to apparent satiety, three times per day from Monday to Saturday (10h00, 12h00, 15h30) and twice on Sundays (10h00, 12h00). Fish mortality and apparent feed intake were recorded daily.

To monitor growth and feed utilization, fish were bulk weighed four weeks after the beginning of the trial under moderate anaesthesia. At the end of the trial, 11 fish from each tank were euthanised with a lethal dose of anaesthetic (1.5 mL L^{-1} 2-phenoxyethanol; Sigma-Aldrich, Spain) and individually weighed and measured. Five fish were pooled and frozen at − 20 ºC for analysis of whole-body composition. From other three fish, viscera and liver were carefully sampled and weighed for determination of somatic indices and a skin-on fillet (fish muscle) was sampled and frozen at − 20 ºC for analysis of protein and lipid contents. In addition, three fish were sampled for analysis of digestive enzyme activity. The digestive system of these fish was carefully dissected and divided into four segments: stomach and pyloric caeca (S/PC), hepatopancreas (HP), anterior/mid intestine (A/MI) and mid/posterior intestine (M/PI). All the above-mentioned regions were carefully weighed, snap-frozen in liquid nitrogen, and kept at − 80 ºC. The remaining fish in each tank were bulk weighed and counted. All samplings were done within 24 h following the last meal.

2.4. Digestive enzyme activity analysis

Samples for analysis of digestive enzyme activity were freeze-dried and shipped to IRTA facilities (Sant Carles de la Ràpita, Spain) where the activity of digestive enzymes was assayed by means of standard biochemical methods. In particular, the activity of pancreatic enzymes like total alkaline proteases, trypsin, α-amylase and bile salt-activated lipase were measured in S/PC, A/MI, M/PI and HP segments, whereas pepsin was measured in the S/PC section. Both intestinal brush border enzymes, alkaline phosphatase and maltase, were measured in A/MI and M/PI segments. Enzyme extracts were prepared considering tissue wet weight, whereas spectrophotometric analyses were performed as recommended by Solovyev and Gisbert (2016) in order to prevent sample deterioration. For the analysis of pancreatic and gastric enzymes, S/PC, A/MI, M/PI and HP samples were homogenized in three volumes (wet weight; w/v) of distilled water at 4 ºC for 1 min, the homogenate was centrifuged at 9 000 x g for 10 min at 4 ºC, and the supernatant was recovered for analytical purposes. Intestinal (A/MI and M/PI) samples for analysis of brush border enzymes were homogenized in 30 volumes (w/v) of ice-cold mannitol (50 mM), Tris-HCl buffer (2 mM), pH 7.0 as described in Gisbert et al. (2018). All analyses were conducted at an individual level (n = 3 per tank; n = 9 per diet). The activity of total alkaline proteases was measured using azocasein (0.5%) as substrate in 0.05 mM Tris-HCl (pH 9.0). One unit (U) of activity was defined as the nmoles of azo dye released per min and per mL of tissue homogenate (λ = 366 nm; García-Carreño and Haard, 1993). Trypsin activity was assayed using BAPNA (N-benzoyl-DL-arginine p-nitroanilide) as substrate; one unit of trypsin per mL (U) was defined as 1 μmol BAPNA hydrolysed per min per mL of enzyme extract (λ = 407 nm; Holm et al., 1988). Regarding α-amylase, its activity (U) was measured using 0.3% soluble starch as substrate and defined as the amount of starch (mg) hydrolysed during 30 min per mL of homogenate (λ = 580 nm; Métais and Bieth, 1968). Bile salt-activated lipase activity was assayed for 30 min using p-nitrophenyl myristate as substrate, and its activity (U) was defined as the amount (nmol) of substrate hydrolysed per min per mL of enzyme extract (λ = 405 nm; Iijima et al., 1998). Pepsin was quantified using 2% haemoglobin as substrate in 1 N HCl buffer, and its activity (U) was defined as the nmol of tyrosine liberated per min per mL of tissue homogenate (λ = 280 nm; Nolasco-Soria et al., 2020). Alkaline phosphatase was quantified using 4-nitrophenyl phosphate (PNPP) as a substrate. One unit (U) was defined as 1 μmol of p-nitrophenol (pNP) released per min per mL of brush border homogenate (λ = 407 nm; Gisbert et al., 2018). Maltase activity was determined using d (+)-maltose as substrate in 100 mM sodium maleate buffer (pH 6.0). One unit of maltase (U) was defined as μmol of glucose liberated per min per mL (λ = 420 nm; Dahlqvist, 1970). Soluble protein of crude enzyme extracts was quantified by means of Bradford's method using bovine serum albumin as standard (Bradford, 1976). All enzymatic activities were measured at 25–26 ºC and expressed as specific activity defined as U mg^{-1} protein. All the assays were made in triplicate (methodological replicates) for each tank and the absorbance was read using a spectrophotometer (Tecan™ Infinite M200, Männedorf Switzerland).

2.5. Chemical analysis

Diet samples were ground until a homogeneous powder was obtained. Whole-body (5 fish per tank) and fish muscle (3 samples per tank) were pooled together by replicate tank and ground until the sample was homogeneous (n = 3 per tank; n = 9 per diet). Diet, fish, and faeces samples were freeze-dried. Chemical analysis (dry matter, ash, crude protein, crude lipids, and gross energy) of samples were performed following standard procedures of the Association of Official Analytical Chemists (AOAC, 2006), as described in Teodósio et al. (2021). Total phosphorus and chromium oxide contents were analysed according to Bolin et al. (1952), as described in Teodósio et al. (2021).

Experimental diets and faeces were analysed for total amino acid content after acid hydrolysis (6 M HCl at 116 °C for 48 h in nitrogen-flushed glass vials). All the samples were then pre-column derivatised with Waters AccQ Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) using the AccQ Tag method (Waters, USA). Analyses were done by ultra-high-performance liquid chromatography (UPLC) in a Waters reversed-phase amino acid analysis system, using norvaline as an internal standard (Aragão et al., 2020).

2.6. Key performance indicators (KPIs)

Indicators of growth performance, feed utilization and nutrient retention were calculated as follows:

Weight gain (%) = 100 x (wet weight gain × initial body weight^{-1}), where wet weight gain is: (final body weight – initial body weight).

Thermal growth coefficient (TGC) = 100 x (final body weight$^{1/3}$ – initial body weight$^{1/3}$) x (Σ degree days)$^{-1}$.

Condition factor (K) = 100 x (body weight × total length^{-3}).

Hepatosomatic index (HSI, %): 100 x (liver weight × body weight^{-1}).

Viscerosomatic index (VSI, %): 100 x (viscera weight × body weight^{-1}).

Daily voluntary feed intake (VFI, % d^{-1}): 100 x (apparent feed intake x ABW^{-1} x days^{-1}), where ABW is average body weight: (final body weight + initial body weight)/2.

Feed conversion ratio (FCR): apparent feed intake x wet weight gain^{-1}.

Protein efficiency ratio (PER): wet weight gain × crude protein intake^{-1}.

Digestible protein, lipid, or energy intake (DPI, DLI or DEI, g or kJ kg^{-1} d^{-1}) = (crude protein, crude lipid, or gross energy intake x ADC% of protein, lipid, or energy) x ABW^{-1} x days^{-1}.

Protein, lipid, or energy retention efficiency (PRE, LRE or ERE, %) = 100 x (final whole-body protein, lipid, or energy content – initial whole-body protein, lipid, or energy content) x (crude protein, crude lipid, or gross energy intake x ADC% of protein, lipid, or energy)$^{-1}$.

Crude nitrogen (N) or phosphorus (P) intake (mg N or P kg^{-1} d^{-1}): N or P intake x ABW^{-1} x days^{-1}.

Nitrogen (N) or phosphorus (P) gain (mg N or P kg^{-1} d^{-1}): (final whole-body N or P content – initial whole-body N or P content) x ABW^{-1} x days^{1}.

Faecal N or P losses (mg N or P kg^{-1} d^{-1}): crude N or P intake x ADC % of N or P.

Metabolic N or P losses (mg N or P kg^{-1} d^{-1}): crude N or P intake - N or P gain - faecal N or P losses.

2.7. Statistical analysis

Data were expressed as means ± standard deviation. Results expressed as percentages were transformed (arcsine square root) before statistical analysis (Ennos, 2007). Data were checked for normal distribution and homogeneity of variances with Levene's test before analysis, if homogeneity of variances was verified an independent sample t-test was used, if homogeneity of variances was not verified, a non-parametric test (Mann-Whitney U test) was performed. Differences were considered significant when $P < 0.05$. All statistical tests were performed using the software program SPSS 25.0 (SPSS Inc. Chicago, IL, USA).

3. Results

3.1. Diet digestibility

Nutrient and energy ADCs were affected by dietary protein to lipid ratios (Table 2). Dry matter and energy digestibility were significantly reduced ($p < 0.05$) by a decrease in dietary lipid content, and a slightly but significantly decrease in lipid ADC was also observed. Protein and phosphorous ADCs were not significantly affected ($p > 0.05$) by dietary treatments. Furthermore, ADC values for all indispensable amino acids were significantly lower ($p < 0.05$) in the LF than in the MF treatment (Fig. 1).

3.2. Growth performance, feed utilization, and activities of digestive enzymes

Fish growth performance was significantly affected by the dietary protein to lipid ratio (Table 3). At the end of the experiment, fish fed the MF diet were significantly heavier ($p < 0.05$) than fish from the LF treatment. Weight gain was significantly higher in fish fed the MF diet than in LF fed fish ($p < 0.05$), but TGC was not significantly different between treatments ($p > 0.05$). Dietary protein to lipid ratio did not affect feed intake, FCR and PER values (Table 3; $p > 0.05$). Digestible protein and energy intakes were similar between fish fed both diets. Nevertheless, fish fed the LF diet had a significantly lower ($p < 0.05$) digestible lipid intake than fish from the MF treatment. Furthermore, the dietary treatments did not affect protein, lipid, or energy retention efficiencies ($p > 0.05$), although lipid retention efficiency was very high in both treatments.

Fish condition factor and VSI were not significantly affected ($p > 0.05$) by the dietary protein to lipid ratio. However, HSI was significantly lower in fed the LF diet than in MF fed fish ($p < 0.05$). Survival was similar among experimental groups ($p > 0.05$).

Concerning the digestive enzymes assayed in the current study, the activity of pepsin in the stomach was similar between treatments (Fig. 2; $p > 0.05$). When considering the activity of pancreatic digestive enzymes, the activity of all assayed enzymes was similar regardless of the diet and the tissue considered (Fig. 3; $p > 0.05$). Regarding the activity of brush border enzymes, no differences in alkaline phosphatase and maltase were found between dietary groups regardless of the intestinal segment (A/MI and M/PI) considered (Fig. 4; $p > 0.05$).

3.3. Fish composition and nutrient balance

Whole-body fish composition at the end of the experiment was significantly affected by the dietary protein to lipid ratios (Table 4). Moisture content was significantly higher ($p < 0.05$), whilst lipid and energy contents were significantly lower in fish fed the LF diet compared

Table 2
Apparent digestibility coefficients (ADC) of nutrients and energy of the experimental diets.

ADC (%)	MF	LF
Dry matter	63.7 ± 4.5^{a}	53.2 ± 0.9^{b}
Protein	92.3 ± 0.8	91.1 ± 0.4
Lipids	92.6 ± 0.7^{a}	91.0 ± 0.5^{b}
Energy	82.2 ± 1.9^{a}	76.5 ± 1.8^{b}
Phosphorus	69.7 ± 3.1	68.7 ± 2.3

Values are presented as means ± standard deviation (n = 3). Different superscripts within the same row indicate significant differences ($p < 0.05$) between treatments. Absence of superscripts indicates no significant differences.

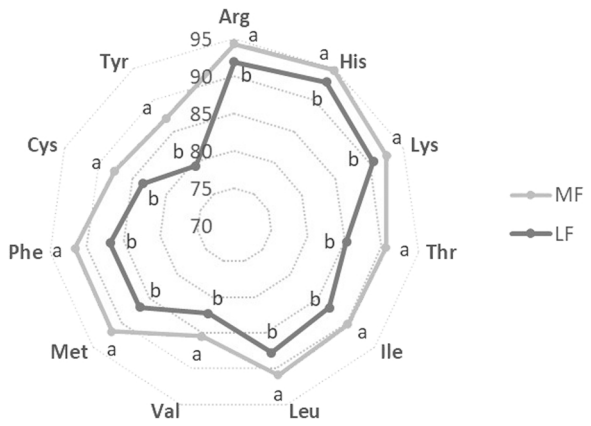

Fig. 1. Apparent digestibility coefficients (ADC) of indispensable amino acids of the experimental diets. Values are presented as means ± standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$) between treatments.

Table 3
Growth performance, feed utilization and somatic indices of gilthead seabream fed diets with different lipid contents (MF: 16% or LF: 12%) for 64 days.

	MF	LF
Final weight (g)	268.9 ± 28.9[a]	249.8 ± 33.9[b]
Weight gain (% IBW)	167 ± 18[a]	151 ± 2[b]
TGC	0.123 ± 0.004	0.112 ± 0.006
VFI (% day^{-1})	1.9 ± 0.1	2.0 ± 0.1
FCR	1.3 ± 0.2	1.4 ± 0.1
PER	2.0 ± 0.2	1.8 ± 0.1
DPI (g kg^{-1} d^{-1})	6.7 ± 0.4	7.0 ± 0.2
DLI (g kg^{-1} d^{-1})	2.8 ± 0.2[a]	2.2 ± 0.1[b]
DEI (kJ kg^{-1} d^{-1})	306 ± 18	288 ± 8
PRE (%)	34.5 ± 3.3	33.0 ± 1.7
LRE (%)	108.1 ± 9.4	120.3 ± 3.3
ERE (%)	55.6 ± 6.1	54.4 ± 1.1
K	1.9 ± 0.1	1.9 ± 0.1
HSI (%)	2.8 ± 0.3[a]	2.6 ± 0.2[b]
VSI (%)	7.9 ± 0.9	7.5 ± 1.1
Survival (%)	98 ± 0	99 ± 1

IBW, initial body weight; TGC = thermal growth coefficient; VFI, daily voluntary feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; DPI, digestible protein intake; DLI, digestible lipid intake; DEI, digestible energy intake; PRE, protein retention efficiency; LRE, lipid retention efficiency; ERE, energy retention efficiency; K, condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index. Initial body weight = 100 ± 7 g. Values are presented as means ± standard deviation (n = 33 for final weight and K; n = 18 for HSI and VSI; n = 3 for the remaining parameters). Different superscripts within the same row indicate significant differences ($p < 0.05$) between treatments. Absence of superscripts indicates no significant differences.

with those fed the MF diet. No significant differences ($p > 0.05$) were found in whole-body protein, ash, and phosphorus contents between experimental groups. Furthermore, the dietary treatments did not affect protein and lipid composition of fish muscle (Table 4; $p > 0.05$).

Data from whole-body fish composition analysis combined with the ADCs of nutrients, allowed the calculation of nitrogen (Fig. 5) and phosphorous (Fig. 6) balances. Daily nitrogen gain (Fig. 5) had mean values of 370 mg N kg^{-1} d^{-1} and was not significantly affected by the dietary treatments. Fish fed with the LF diet had significantly higher ($p > 0.05$) faecal nitrogen losses (109 ± 3 mg N kg^{-1} d^{-1}) than fish fed the MF diet (90 ± 5 mg N kg^{-1} d^{-1}). Metabolic nitrogen losses ranged from 707 ± 76 mg N kg^{-1} d^{-1} in fish fed with the MF diet to 752 ± 37 mg N kg^{-1} d^{-1} in LF fed fish and were not significantly affected ($p > 0.05$) by the dietary treatments. When considering the total nitrogen losses, the results were not significantly affected by the dietary protein to lipid ratio ($p > 0.05$).

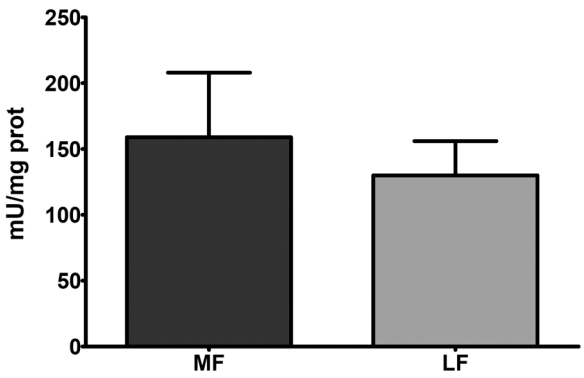

Fig. 2. Activity of pepsin in the stomach and pyloric caeca (S/PC) of gilthead seabream fed diets with different lipid contents (MF: 16% or LF: 12%) for 64 days. Values are presented as means ± standard deviation (n = 9). Absence of letters indicates no significant differences ($p > 0.05$) between treatments.

Concerning the phosphorus balance (Fig. 6), the dietary treatments had no effect on daily phosphorus gain or on faecal and metabolic phosphorus losses ($p > 0.05$). Phosphorus gain ranged from 60 ± 13 mg P kg^{-1} d^{-1} in the MF treatment to 64 ± 13 mg P kg^{-1} d^{-1} in the LF treatment, while total phosphorus losses ranged from 88 ± 12 mg P kg^{-1} d^{-1} to 92 ± 17 mg P kg^{-1} d^{-1} in fish fed with the MF and the LF diets, respectively.

4. Discussion

Previous works concluded that feeding gilthead seabream during the on-growing phase with practical diets containing low levels of marine-derived proteins (13%) and a mixture of fish and vegetable oils did not affect growth performance (Dias et al., 2009). Moreover, diets with 16–18% lipid content were identified as the most suitable to farm seabream during summer conditions (Bonaldo et al., 2010; Mongile et al., 2014). Therefore, due to the well-identified problem of fat accumulation in seabream during summer (Grigorakis et al., 2002; Pleadin et al., 2015), this study intended to test if a reduction in fat content in contemporary diets could improve fish performance and quality during this period.

A reduction in dietary lipid content decreased the dry matter, lipid, and energy digestibility, as well as the ADCs of all the indispensable amino acids. ADCs of nutrients and energy were within the values previously observed for this species using the same methodology (Aragão et al., 2020; Dias et al., 2009). Previous works with gilthead seabream reported some effects of dietary lipid level on nutrient digestibility. Santinha et al. (1999) verified that a decrease in dietary lipid content from 19.5% to 14% reduced dry matter digestibility but without any effects on lipid, protein, phosphorus, or energy ADCs. It should be noted that in the current study the decrease in dietary lipid content was compensated by a small increase in wheat meal and by the addition of cellulose, which is not digested by the fish (Dabrowski and Guderley, 2002). Dietary cellulose inclusion has been shown to decrease the ADC of dry matter and energy (Ren et al., 2015), but at the levels used in the current experiment (2%) no major impact on nutrient digestibility was expected. Fountoulaki et al. (2005) reported a decrease in fat digestibility in gilthead seabream when lowering the dietary lipid level from 20% to 10%, although the main effect of this decrease was associated with the increase of dietary starch (from 25% to 36%). Therefore, the reduction in lipid and energy ADCs in the current study is probably linked to the lower dietary lipid inclusion and not to the slight increase in carbohydrate content. Contrarily to the diet used in this study, in the above-mentioned studies fish oil was the only lipid source and none or very low levels of plant ingredients were included. In seabream fed diets with low inclusion of marine-derived proteins, and in particular, when fish oil was partially replaced by vegetable oils, lipid digestibility was

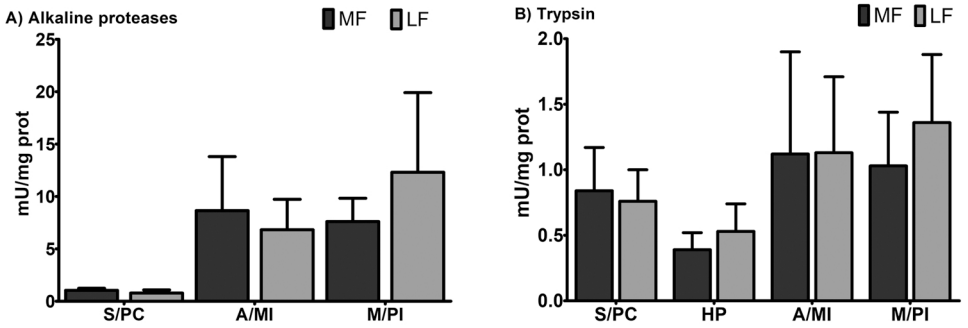

Fig. 3. Activities of pancreatic (A: total alkaline proteases, B: trypsin, C: α-amylase and D: bile salt-activated lipase) enzymes in the stomach and pyloric caeca (S/PC), hepatopancreas (HP), anterior/mid intestine (A/MI) or mid/posterior intestine (M/PI) of gilthead seabream fed diets with different lipid contents (MF: 16% or LF: 12%) for 64 days. Values are presented as means ± standard deviation (n = 7–9). Absence of letters indicates no significant differences ($p > 0.05$) between treatments.

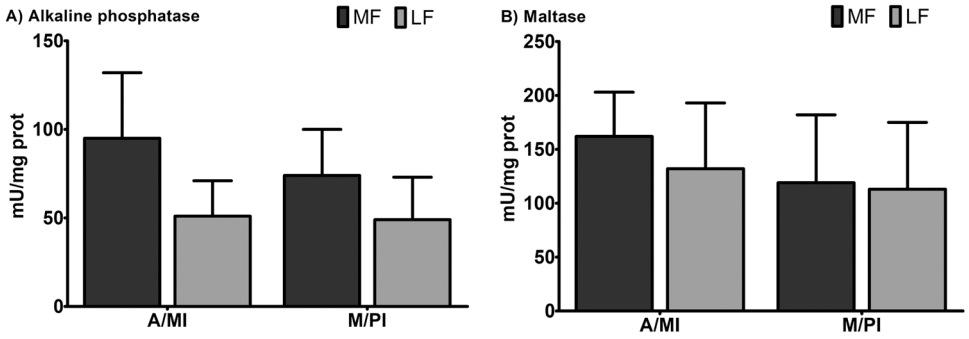

Fig. 4. Activities of the brush border enzymes (A - alkaline phosphatase and B - maltase) in anterior/mid intestine (A/MI) and mid/posterior intestine (M/PI) of gilthead seabream fed diets with different lipid contents (MF: 16% or LF: 12%) for 64 days. Values are presented as means ± standard deviation (n = 8–9). Absence of letters indicates no significant differences ($p > 0.05$) between treatments.

Table 4
Whole-body and muscle composition of gilthead seabream fed diets with different lipid contents (MF: 16% or LF: 12%) for 64 days.

Fish composition	MF	LF
Whole-body (% wet weight)		
Moisture	61.7 ± 0.1[b]	62.7 ± 0.7[a]
Ash	1.2 ± 0.1	1.2 ± 0.4
Protein	16.4 ± 0.3	16.9 ± 0.4
Lipids	17.4 ± 0.3[a]	16.4 ± 0.2[b]
Phosphorous	0.5 ± 0.1	0.5 ± 0.1
Energy (MJ kg^{-1})	10.5 ± 0.1[a]	10.2 ± 0.1[b]
Muscle (% wet weight)		
Protein	21.2 ± 0.6	21.3 ± 0.8
Lipids	15.1 ± 0.5	14.4 ± 0.1

Values are presented as means ± standard deviation (n = 3). Different superscripts within the same row indicate significant differences ($p < 0.05$) between treatments. Absence of superscripts indicates no significant differences.

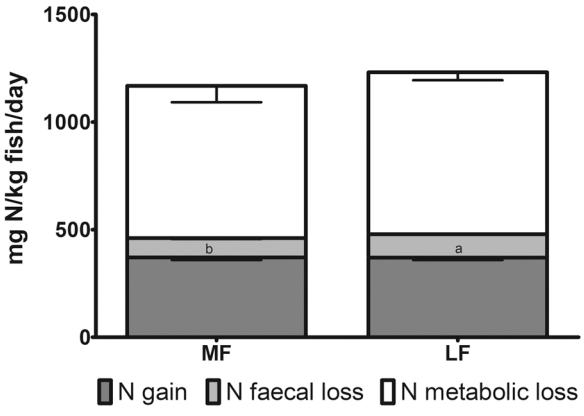

Fig. 5. Daily nitrogen (N) balance in gilthead seabream fed diets with different lipid contents (MF: 16% or LF: 12%) for 64 days. Values are presented as means ± standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$) between treatments among the same fraction. Absence of letters indicates no significant differences.

negatively affected (Dias et al., 2009). As for the amino acids, it has been previously observed that a decrease in dietary lipid content may negatively affect amino acid digestibility (Teodósio et al., 2022). A decrease in dietary lipid levels may result in a faster intestinal transit, thus

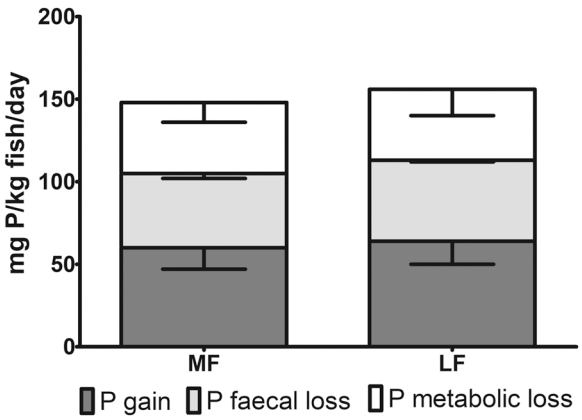

Fig. 6. Daily phosphorus (P) balance in gilthead seabream fed diets with different lipid contents (MF: 16% or LF: 12%) for 64 days. Values are presented as means ± standard deviation (n = 3). Absence of letters indicates no significant differences ($p > 0.05$) between treatments among the same fraction.

decreasing amino acid uptake efficiency. The intestinal uptake of some amino acids has been shown to increase with an increase in dietary lipid content (García-Meilán et al., 2016). The lower ADC of all indispensable amino acids found for the LF diet resulted in higher nitrogen faecal losses in fish from this treatment. This indicates that in a low fishmeal and fish oil scenario, low dietary lipid levels have a detrimental effect on lipid and amino acid digestibility, and consequently on energy digestibility, thus increasing the nitrogen losses to the environment.

The activity of digestive enzymes plays an important role in determining the digestibility of nutrients. However, in this study, the lower ADCs found in the LF diet were not related to dietary effects on the digestive enzymes, as no differences in their activities were found between experimental groups. The dietary fat level has been shown to differently affect the digestive enzymes in seabream, in particular, a clear effect on α-amylase levels in the digestive tract was observed, while the effects on protease activity were of smaller magnitude (Fountoulaki et al., 2005), and no effects were found in lipase activity (Mongile et al., 2014). On the contrary, in European seabass (*Dicentrarchus labrax*), activities of α-amylase and trypsin were not affected by dietary CL levels, like in the current study, while the lipase activity increased with a decrease in dietary lipids (García-Meilán et al., 2016). In spotted seabass (*Lateolabrax maculatus*) fed diets with different protein to lipid ratios, intestinal protease, amylase, and lipase activities were not affected (Lu et al., 2020), as observed in the current study with gilthead seabream. Such differences between studies may not be attributed just to changes in dietary lipids, as the overall composition of experimental diets, from qualitative and quantitative points of view, should be considered. Thus, extracting conclusions from the literature on the impact of dietary CL levels on the activity of digestive enzymes is not as straightforward as it is for other variables since nutritional studies are barely comparable. Therefore, results from the present study indicate that changes in protein to lipid ratio, considering the ingredients used in diet formulation, did not modulate the activity of gastric, pancreatic and brush border intestinal enzymes in seabream on-grown during summer conditions.

Previously, it was shown that a decrease in dietary lipid content to 16% (in diets with 45–47% CP) did not affect the growth performance of gilthead seabream during summer (Bonaldo et al., 2010; Mongile et al., 2014). However, at the low protein inclusion levels used in this study and with fish close to the market size, a further reduction in dietary CL affected the growth performance. This is of special relevance since the reduced amino acid digestibility in seabream fed with the LF diet possible contributed to the lower growth performance of these fish when compared with fish fed the MF diet, as feed utilization was only slightly affected by the dietary lipid content. The FCR was similar between treatments and can be considered in the same range as in previous studies performed with seabream during summer (Bonaldo et al., 2010; Mongile et al., 2014). In those previous studies, a reduction in dietary lipid levels from 24-32% to 16% affected the FCR. Hence, the present results indicate that a plateau could have been reached if dietary lipid content is further reduced. Similarly to other studies performed during summer conditions (Bonaldo et al., 2010; Velázquez et al., 2006), the dietary protein to lipid ratios had no influence on PER and on protein retention efficiency. However, the slightly reduced lipid digestibility found for the LF diet was translated into a significantly lower digestible lipid intake. Nevertheless, lipid retention efficiency was high and without significant differences between treatments. Irrespectively of the dietary protein and lipid contents, one-fifth of the digestible protein supplied to seabream was shown to be converted into body lipid (Ekmann et al., 2013). Furthermore, gross lipid efficiency and lipid retention efficiency in seabream were significantly increased with a decrease in dietary lipid content (Bonaldo et al., 2010; Mongile et al., 2014; Velázquez et al., 2006). This growth trial was performed during a period when cultured seabream is known to considerably increase the whole-body lipid content (Pleadin et al., 2015), which allied to the low/medium dietary lipid levels used may explain the high lipid retention efficiency found in both treatments.

In general, whole-body lipid content was high in both dietary treatments, in accordance with previous results found for seabream reared during the same period of the year (Bonaldo et al., 2010; Mongile et al., 2014; Pleadin et al., 2015; Velázquez et al., 2006). Nevertheless, the reduction in the dietary lipid level significantly decreased the whole-body lipid content, as similarly observed for several Mediterranean fish species reared during summer (Adamidou et al., 2011; Chatzifotis et al., 2010; Velázquez et al., 2006). This effect was not observed in seabream during winter conditions (Velázquez et al., 2006). Still, other studies performed in the summer showed that a reduction in dietary lipid levels from 24-32% to 16% did not promote a decrease in whole-body lipid content (Bonaldo et al., 2010; Mongile et al., 2014). Additionally, the dietary protein to lipid ratios had no effect on the whole-body protein content, as previously observed in seabream and other Mediterranean fish species under summer conditions (Adamidou et al., 2011; Bonaldo et al., 2010; Chatzifotis et al., 2010; Mongile et al., 2014; Velázquez et al., 2006). In salmonids, it was shown that the body protein content is endogenously controlled, while the lipid level is controlled by endogenous and exogenous factors (Shearer, 1994). In fact, protein content in seabream is relatively stable even throughout the seasons, contrarily to what is observed for the lipid content (Grigorakis et al., 2002; Pleadin et al., 2015). These results indicate a potential benefit of the LF diet towards reducing fat accumulation in seabream during summer.

Muscle composition analysis showed no significant differences between treatments, similarly to what was observed in previous studies with seabream and seabass reared during similar conditions (Katsika et al., 2021; Mongile et al., 2014). Since the whole-body lipid content significantly decreased in fish fed the LF diet, this result suggests a reduction in the visceral fat, which seems to be corroborated by the decrease in HSI and the lower numerical value of VSI found in this dietary treatment. The accumulation of perivisceral and peritoneal fat, especially in summer, is well-known in farmed seabream and one of the potential quality problems faced by the industry (Grigorakis et al., 2002; Pleadin et al., 2015). Despite the interesting results obtained when considering fish quality, the decrease in dietary lipid content resulted in a slight impairment in fish growth, which depending on the production cycle may result in economic impacts, since fish need to stay longer in the tanks/cages until the commercial size is attained.

Diet formulations need to carefully consider lipid level inclusion, so the market value of the fish is not reduced. Nevertheless, this should not disregard the environmental impact. The dietary protein to lipid ratios had no effect on the phosphorus balance and phosphorus losses were relatively low, as previously observed in seabream fed high-plant diets (Aragão et al., 2020; Dias et al., 2009). Thus, no detrimental effects of

one diet compared to the other in terms of the phosphorus discharges to the environment were observed. However, as previously discussed, the reduction in dietary CL resulted in higher nitrogen faecal losses to the environment in fish fed the LF diet, due to a negative effect on amino acid digestibility. The total nitrogen losses were not significantly different between treatments, but high numerical values were found for fish fed with the LF diet. Therefore, the environmental impact of the nitrogen losses during seabream on-growing should be taken into consideration when estimating the sustainability of the production.

In conclusion, the reduction in dietary lipid content in a contemporary diet decreased the whole-body lipid content in seabream juveniles reared during summer. However, this diet formulation reduced amino acid digestibility, resulting in a slight growth impairment and in higher nitrogen faecal losses. Ultimately, the choice for an optimal dietary formulation needs to further consider the environmental impacts, especially in scenarios like Mediterranean fish farming, where farmers have no control over water temperature. Understanding the interaction between environmental conditions and feed formulations is of utmost importance to guarantee a sustainable fish production in the Mediterranean.

CRediT authorship contribution statement

Cláudia Aragão: Conceptualization, Methodology, Resources, Validation, Writing - original draft, Writing - review & editing, Funding acquisition, **Miguel Cabano**: Formal analysis, Visualization, Writing - original draft, **Rita Colen**: Investigation, Formal analysis, Writing - original draft, **Rita Teodósio**: Investigation, Formal analysis, **Enric Gisbert**: Resources, Validation, Writing - review & Editing, **Jorge Dias**: Conceptualization, Writing - review & editing, **Sofia Engrola**: Conceptualization, Methodology, Resources, 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.

Acknowledgements

The authors would like to thank Gonçalo Nunes and Raquel Carrilho for their participation in the rearing trials.

This work was supported by European Union's Horizon 2020, European Union research and innovation programme under Project MedAID (GA no 727315) and by the Portuguese Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 to CCMAR and contract DL 57/2016/CP1361/CT0033 to CA.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2022.101262.

References

Adamidou, S., Rigos, G., Mente, E., Nengas, I., Fountoulaki, E., 2011. The effects of dietary lipid and fibre levels on digestibility of diet and on the growth performance of sharpsnout seabream (Diplodus puntazzo. Mediterr. Mar. Sci. 12, 401–412. https://doi.org/10.12681/MMS.40.

AOAC, 2006. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Gaithersburgs, MD, USA.

Aragão, C., Cabano, M., Colen, R., Fuentes, J., Dias, J., 2020. Alternative formulations for gilthead seabream diets: Towards a more sustainable production. Aquac. Nutr. 26, 444–455. https://doi.org/10.1111/anu.13007.

Bolin, D.W., King, R.P., Klosterman, E.W., 1952. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index substance. Science 116, 634–635.

Bonaldo, A., Isani, G., Fontanillas, R., Parma, L., Grilli, E., Gatta, P.P., 2010. Growth and feed utilization of gilthead sea bream (Sparus aurata, L.) fed to satiation and restrictively at increasing dietary energy levels. Aquac. Int. 18, 909–919. https://doi.org/10.1007/s10499-009-9312-0.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Chang, J., Niu, H.X., Jia, Y.D., Li, S.G., Xu, G.F., 2018. Effects of dietary lipid levels on growth, feed utilization, digestive tract enzyme activity and lipid deposition of juvenile Manchurian trout, Brachymystax lenok (Pallas). Aquac. Nutr. 24, 694–701. https://doi.org/10.1111/anu.12598.

Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I., Mylonas, C. C., 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (Argyrosomus regius) juveniles. Aquaculture 307, 65–70. https://doi.org/10.1016/j.aquaculture.2010.07.002.

Company, R., Calduch-Giner, J.A., Pérez-Sánchez, J., Kaushik, S.J., 1999. Protein sparing effect of dietary lipids in common dentex (Dentex dentex): A comparative study with sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax). Aquat. Living Resour. 12, 23–30. https://doi.org/10.1016/S0990-7440(99)80011-4.

Dabrowski, K., Guderley, H., 2002. Intermediary metabolism. In: Halver, J.E., Hardy, R. W. (Eds.), Fish Nutrition. Academic Press, San Diego, USA, pp. 309–365.

Dahlqvist, A., 1970. Assay of intestinal disaccharidases. Enzymol. Biol. Et. Clin. 11, 52–66. https://doi.org/10.1159/000458348.

Dias, J., Conceição, L.E.C., Ribeiro, A.R., Borges, P., Valente, L.M.P., Dinis, M.T., 2009. Practical diet with low fish-derived protein is able to sustain growth performance in gilthead seabream (Sparus aurata) during the grow-out phase. Aquaculture 293, 255–262. https://doi.org/10.1016/j.aquaculture.2009.04.042.

Ekmann, K.S., Dalsgaard, J., Holm, J., Campbell, P.J., Skov, P.V., 2013. Effects of dietary energy density and digestible protein:energy ratio on de novo lipid synthesis from dietary protein in gilthead sea bream (Sparus aurata) quantified with stable isotopes. Br. J. Nutr. 110, 1771–1781. https://doi.org/10.1017/S0007114513001281.

Ennos, R., 2007. Statistical and Data Handling Skills in Biology, 2nd ed. Pearson Prentice Hall, Harlow, England.

Fountoulaki, E., Alexis, M.N., Nengas, I., Venou, B., 2005. Effect of diet composition on nutrient digestibility and digestive enzyme levels of gilthead sea bream (Sparus aurata L.). Aquac. Res. 36, 1243–1251. https://doi.org/10.1111/j.1365-2109.2005.01232.x.

García-Carreño, F.L., Haard, N.F., 1993. Characterization of proteinase classes in langostilla (Pleuroncodes planipes) and crayfish (Pacifastacus astacus) extracts. J. Food Biochem. 17, 97–113. https://doi.org/10.1111/j.1745-4514.1993.tb00864.x.

García-Meilán, I., Ordóñez-Grande, B., Machahua, C., Buenestado, S., Fontanillas, R., Gallardo, M.A., 2016. Effects of dietary protein-to-lipid ratio on digestive and absorptive processes in sea bass fingerlings. Aquaculture 463, 163–173. https://doi.org/10.1016/j.aquaculture.2016.05.039.

Gartzia, I., Peral, I., Alfaro, B., Riesco, S., Santa-Cruz, E., Krystallis, A., Brunso, K., Stancu, V., Claret, A., Guerrero, L., 2018. Identification of product and market requirements of aquaculture chain stakeholders. Deliverable 5.1 of the Horizon 2020 project MedAID (GA number 727315), published in the project web site on 21.06.2018: http://www.medaid-h2020.eu/index.php/deliverables/.

Gisbert, E., Nolasco, H., Solovyev, M., 2018. Towards the standardization of brush border purification and intestinal alkaline phosphatase quantification in fish with notes on other digestive enzymes. Aquaculture 487, 102–108. https://doi.org/10.1016/j.aquaculture.2018.01.004.

Grigorakis, K., 2007. Compositional and organoleptic quality of farmed and wild gilthead sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) and factors affecting it: A review. Aquaculture 272, 55–75. https://doi.org/10.1016/j.aquaculture.2007.04.062.

Grigorakis, K., Alexis, M.N., Anthony Taylor, K.D., Hole, M., 2002. Comparison of wild and cultured gilthead sea bream (Sparus aurata); composition, appearance and seasonal variations. Int. J. Food Sci. Technol. 37, 477–484. https://doi.org/10.1046/j.1365-2621.2002.00604.x.

Halver, J.E., Hardy, R.W., 2002. Nutrient flow and retention. In: Halver, J.E., Hardy, R. W. (Eds.), Fish Nutrition. Academic Press, San Diego, USA, pp. 755–770.

Hernández, J.M., Gasca-Leyva, E., León, C.J., Vergara, J.M., 2003. A growth model for gilthead seabream (Sparus aurata. Ecol. Model. 165, 265–283. https://doi.org/10.1016/S0304-3800(03)00095-4.

Holm, H., Hanssen, L.E., Krogdahl, A., Florholmen, J., 1988. High and low inhibitor soybean meals affect human duodenal proteinase activity differently: In vivo comparison with bovine serum albumin. J. Nutr. 118, 515–520. https://doi.org/10.1093/jn/118.4.515.

Iijima, N., Tanaka, S., Ota, Y., 1998. Purification and characterization of bile salt-activated lipase from the hepatopancreas of red sea bream, Pagrus major. Fish. Physiol. Biochem. 18, 59–69.

Karalazos, V., Bendiksen, E.A., Dick, J.R., Tocher, D.R., Gordon Bell, J., 2011. Influence of the dietary protein:lipid ratio and fish oil substitution on fatty acid composition and metabolism of Atlantic salmon (Salmo salar) reared at high water temperatures. Br. J. Nutr. 105, 1012–1025. https://doi.org/10.1017/S0007114510004605.

Katsika, L., Flores, M.H., Kotzamanis, Y., Estevez, A., Chatzifotis, S., 2021. Understanding the interaction effects between dietary lipid content and rearing temperature on growth performance, feed utilization, and fat deposition of sea bass (Dicentrarchus labrax. Animals 11, 392. https://doi.org/10.3390/ANI11020392.

Li, X., Jiang, Y., Liu, W., Ge, X., 2012. Protein-sparing effect of dietary lipid in practical diets for blunt snout bream (Megalobrama amblycephala) fingerlings: effects on digestive and metabolic responses. Fish. Physiol. Biochem. 38, 529–541. https://doi.org/10.1007/s10695-011-9533-9.

Lu, K.-L., Cai, L.-S., Wang, L., Song, K., Zhang, C.-X., Rahimnejad, S., 2020. Effects of dietary protein/energy ratio and water temperature on growth performance, digestive enzymes activity and non-specific immune response of spotted seabass (Lateolabrax maculatus). Aquac. Nutr. 26, 2023–2031. https://doi.org/10.1111/ANU.13143.

Maestre, R., Pazos, M., Medina, I., 2011. Role of the raw composition of pelagic fish muscle on the development of lipid oxidation and rancidity during storage, 6384-6291 J. Agric. Food Chem. 59. https://doi.org/10.1021/jf200686z.

Maynard, L.A., Loosli, J.K., Hintz, H.F., Warner, R.G., 1979. Animal Nutrition. McGraw-Hill, New York.

Métais, P., Bieth, J., 1968. Détermination de l'α-amylase par une microtechnique. Ann. De. Biol. Clin. 26, 133–142.

Mongile, F., Bonaldo, A., Fontanillas, R., Mariani, L., Badiani, A., Bonvini, E., Parma, L., 2014. Effects of dietary lipid level on growth and feed utilisation of gilthead seabream (Sparus aurata L.) reared at Mediterranean summer temperature. Ital. J. Anim. Sci. 13, 30–34. https://doi.org/10.4081/ijas.2014.2999.

Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Little, D. C., Lubchenco, J., Shumway, S.E., Troell, M., 2021. A 20-year retrospective review of global aquaculture. Nature 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6.

Nolasco-Soria, H., Nolasco-Alzaga, H.R., Gisbert, E., 2020. The importance of pepsin-like acid protease quantification in aquaculture studies: a revision of available procedures and presentation of a new protocol for its assessment. Rev. Aquac. 12, 1928–1943. https://doi.org/10.1111/raq.12417.

Pleadin, J., Petrovic, M., Krešic, G., Zrncic, S., Oraic, D., Džafic, N., 2015. Influence of season and farming location on the quality parameters of sea bass (Dicentrarchus labrax) and sea bream (Sparus aurata). Ital. J. Food Sci. 27, 23–31. https://doi.org/10.14674/1120-1770/ijfs.v181.

Ren, M., Habte-Tsion, H.-M., Xie, J., Liu, B., Zhou, Q., Ge, X., Pan, L., Chen, R., 2015. Effects of dietary carbohydrate source on growth performance, diet digestibility and liver glucose enzyme activity in blunt snout bream, Megalobrama amblycephala. Aquaculture 438, 75–81. https://doi.org/10.1016/j.aquaculture.2015.01.008.

Santinha, P.J.M., Médale, F., Corraze, G., Gomes, E., 1999. Effects of the dietary protein: lipid ratio on growth and nutrient utilization in gilthead seabream (Sparus aurata L.). Aquac. Nutr. 5, 147–156.

seatemperature.org, 2021. Available at: www.seatemperature.org/mediterranean-sea [Online]. Accessed: 1 September 2021.

de la Serrana, D.G., Fontanillas, R., Koppe, W., Fernández-Borràs, J., Blasco, J., Martín-Pérez, M., Navarro, I., Gutiérrez, J., 2013. Effects of variable protein and lipid proportion in gilthead sea bream (Sparus aurata) diets on fillet structure and quality. Aquac. Nutr. 19, 368–381. https://doi.org/10.1111/j.1365-2095.2012.00966.x.

Shearer, K.D., 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 119, 63–88. https://doi.org/10.1016/0044-8486(94)90444-8.

Solovyev, M., Gisbert, E., 2016. Influence of time, storage temperature and freeze/thaw cycles on the activity of digestive enzymes from gilthead sea bream (Sparus aurata. Fish. Physiol. Biochem. 42, 1383–1394. https://doi.org/10.1007/s10695-016-0226-2.

Tacon, A.G.J., Lemos, D., Metian, M., 2020. Fish for health: improved nutritional quality of cultured fish for human consumption. Rev. Fish. Sci. Aquac. 28, 449–458. https://doi.org/10.1080/23308249.2020.1762163.

Teodósio, R., Aragão, C., Colen, R., Carrilho, R., Dias, J., Engrola, S., 2021. A nutritional strategy to promote gilthead seabream performance under low temperatures. Aquaculture 537, 736494. https://doi.org/10.1016/j.aquaculture.2021.736494.

Teodósio, R., Aragão, C., Conceição, L.E.C., Dias, J., Engrola, S., 2022. Amino acid metabolism in gilthead seabream is affected by the dietary protein to energy ratios. Aquac. Nutr. 2022, 8230704. https://doi.org/10.1155/2022/8230704.

Velázquez, M., Zamora, S., Martínez, F.J., 2006. Effect of dietary energy content on gilthead sea bream (Sparus aurata) feeding behaviour and nutritional use of the diet. Aquac. Nutr. 12, 127–133. https://doi.org/10.1111/j.1365-2095.2006.00391.x.

Yigit, M., Yardim, Ö., Koshio, S., 2002. The protein sparing effects of high lipid levels in diets for rainbow trout (Oncorhynchus mykiss, W. 1792) with special reference to reduction of total nitrogen excretion. Isr. J. Aquac. - Bamidgeh 54, 79–88. https://doi.org/10.46989/001c.20314.