Universidade de Lisboa
Faculdade de Ciências
Departamento de Biologia Animal

Optimizing the dietary protein:lipid ratio on meagre (*Argyrosomus regius*): effects on growth and lipid deposition

João Paulo Cardoso Lopes Fernandes

Dissertação
Mestrado em Ecologia Marinha

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RESUMO

A aquicultura é um dos tipos mais modernos de agricultura praticada no Mundo, sendo uma das respostas para a crescente demanda por produtos de origem animal e seus derivados. Devido à baixa diversidade de espécies produzidas na Europa, surgiu a necessidade de diversificar os produtos atualmente produzidos, com a implementação de novas espécies passíveis de serem criadas a um nível industrial. Nesse contexto, foi proposta a implementação da criação e produção de corvina (*Argyrosomus regius*), por apresentar características necessárias a uma produção sustentável: requisitos biológicos facilmente atingíveis (crescimento, fecundidade e criação em cativeiro), requisitos tecnológicos (índice de conversão apropriado, ciclo de vida bem conhecido) e requisitos de mercado (penetração de mercado, alto valor comercial e disponibilidade anual), que, em conjunto com um custo de produção reduzido, podem competir com as espécies já cultivadas.

A corvina faz parte da família Sciaenidae, sendo uma das maiores famílias de peixes roncadores, incluindo 270 espécies dentro de 70 géneros. São peixes roncadores, porque emitem sons durante a época de desova. Possuem uma ampla distribuição entre as regiões temperadas e tropicais do Mundo, incluindo Portugal, de Norte a Sul. Atualmente já existem sete espécies cultivadas a nível comercial e/ou experimental, das quais a corvina faz parte. A corvina é um peixe teleósteo, sendo um dos maiores membros da família Sciaenidae. Pode crescer mais de 180 cm de comprimento total e peso superior a 50 kg. Tem uma ampla distribuição, estando presente nos mares Mediterrâneo e Negro, na costa Atlântica da Europa e costa oeste africana, onde vivem em estuários e águas costeiras, próximo do fundo ou perto da superfície (profundidades variam de 15 a 200 m). A qualidade da carne é de alto valor nutricional, dando origem ao seu nome latino *regius*, para qualidade "real" da sua carne. O ciclo de vida já está fechado, com muitas "*hatcheries"* espalhadas por toda a Europa, sendo a engorda feita com sucesso em jaulas ou tanques (prática realizada nos países do Mediterrâneo, como Grécia, França, Itália, Espanha e Egito). Apesar das técnicas para as diferentes fases de criação estarem bem estabelecidas, a produção ainda não atingiu o seu potencial pleno, principalmente devido aos protocolos alimentares, uma vez que estes são os mesmos utilizados em outras espécies, com requisitos bem conhecidos em termos de nutrientes. É um peixe carnívoro, que, com a alimentação apropriada, consegue atingir 1 kg em menos de um ano (cultivo em jaulas), pelo que um rácio proteína:lípido apropriado será a resposta para esse elevado crescimento.

Relativamente à formulação de uma dieta apropriada, o valor de proteína alimentar deverá ser superior a 45%, e o valor de lípidos não superior a 17%, em termos de ração total. Os aminoácidos e os ácidos gordos essenciais fornecidos nas rações permitem criar balanços energéticos que irão sustentar o rápido crescimento da corvina. Os ácidos gordos são colocados nas rações através de óleos de peixe, que contêm ácidos gordos saturados, monoinsaturados e polinsaturados, sendo estes últimos aqueles que possuem efeitos benéficos para o ser humano – ómega-3. Os ácidos eicosapentaenóico (EPA) e docosahexaenóico (DHA) são os principais ácidos gordos fornecidos pelos óleos de peixe, e essenciais a todos os peixes. Hidratos de carbono são utilizados na formulação das rações, servindo como uma fonte de uso

limitada para produção de energia, bem como para dar forma e estabilidade às rações. Minerais e vitaminas também são introduzidos nas rações, para suplementar deficiências a esse nível.

Para averiguar quais os níveis ótimos de proteínas e lípidos a incluir nas rações de corvina, e averiguar como esses nutrientes são utilizados, foi realizado um ensaio experimental, com duração total de 12 semanas.

Cinco dietas isoenergéticas (21,92±0,57 MJ/kg) (média±desvpad) foram criadas, com variações nos níveis de proteína (44% e 50%) e lípidos (12%, 15% e 18%), sendo denominadas de 44L15, 44L18, 50L12, 50L15 e 50L18. O ensaio decorreu desde Setembro/2011 a Dezembro/2011 (63 dias), com um número inicial de 945 peixes (63,66±2,78 g), distribuídos aleatoriamente por 15 tanques de fibra de vidro (volume total de 1500L), em grupos triplicados. Os peixes foram aclimatados durante uma semana, e o ensaio começou posteriormente. Os peixes eram alimentados diariamente, às 9h00 e às 16h (dias de semana) e às 9h e às 13h (fins de semana), sendo a alimentação feita à mão, *ad libitum* (até à saciação).

Foram realizadas quatro amostragens: uma inicial, duas intermédias, e uma final. Em todas houve verificação do peso corporal e tamanho, sendo que na final foram sacrificados 12 peixes, dos quais 6 foram usados para a análise corporal proximal, e outros 6 para análise do conteúdo lipídico das amostras de músculo e fígado (lípidos totais e perfil de ácidos gordos). A análise estatística foi realizada com recurso ao software STATISTICA 11.0, onde os dados foram sujeitos à análise de variância (ANOVA), com p <0,05 e quando normalidade e homocedasticidade eram cumpridas. Caso contrário, testes não paramétricos – análise de variância Kruskal-Wallis – eram realizados para averiguar diferenças.

No geral, e tendo em conta os parâmetros de crescimento calculados, a dieta 50L18 apresentou o melhor desempenho, quando comparada com as outras dietas, uma vez que possui os níveis desejados de proteína e de lípidos, com proteína > 45% e lípidos ≈ 17%. Por outro lado, a alimentação 50L12 teve o pior desempenho, principalmente por causa dos baixos níveis de lipídios na dieta. As taxas de SGR (taxa de crescimento específico) devem ser maiores do que 1% / dia, situação alcançada com as dietas 50L15 e 50L18 que possuíam os SGR mais elevados, de 0,99±0,04 e 1,21±0,00, respetivamente. Dentro do grupo com 50% de proteína, a dieta 50L18 apresentou a maior Taxa de Crescimento Diário (DGI): 1,78±0,03 %, a maior Eficiência da Ração (FE): 0,97±0,04, e o maior Rácio de Eficiência Proteica (PER): 2,33±0,09, possuindo o menor valor médio de Rácio de Conversão Alimentar (FCR): 1,04±0,04. O valor de DGI obtido é devido aos elevados valores de proteína e lípido presentes na ração, que acabaram por se refletir num peso final superior aos outros tratamentos. O valor de PER está relacionado com valores elevados de FE, bem como de peso final elevado, situação que se confirmou com esta dieta, visto que os peixes que cresceram mais foram aqueles que se alimentaram mais. O FCR ao ser mais baixo que nas outras dietas indica que, com a mesma quantidade de alimento, a conversão da dieta 50L18 em elementos bio disponíveis é feita com maior eficácia. A dieta 50L18 também foi aquela que apresentou os valores médios mais elevados para a retenção de proteína (42,24±2,50 **%**) e energia (26,98±0,47 %), bem como para os valores médios de ganho diário de nitrogénio (N) (334,56±8,26 mg/kg/dia) e energia (0,71±0,04 kJ/kg/dia). Com estes valores, fica confirmado que dietas com valores aproximados de

50% proteína são essenciais para o crescimento da corvina. A dieta 50L12 apresentou o pior valor de ganho diário de energia (0,51±0,16 kJ/kg/dia), o que indica que não será apropriada para o cultivo de corvina, inclusive por apresentar uma retenção de lípidos (17,65±13,01 %) bastante inferior aos outros tratamentos.

Relativamente à composição proximal dos peixes alimentados com cada dieta experimental, em termos de matéria seca, a dieta 44L18 foi a que apresentou o menor valor médio de proteína (64,97±2,32 %) em oposição à dieta 50L12 (69,94±3,11 %), sendo o valor de lípidos o que mais influencia o valor de proteína presente. A dieta 50L12 apresentou o menos valor médio de lípidos (16,78±2,86 %), sendo ainda menos do que o inicial (19,63±0,14 %), o que indica a má performance desta dieta. Em termos de matéria fresca, as dietas do grupo de 50% proteína possuíram melhor performance que a dieta 44L18, sendo que esta, numa base de matéria fresca de lípidos e de energia, obteve os valores médios mais elevados (6,53±0,94 % e 6,44±0,29 % respetivamente), visto possuir uma elevada concentração de lípidos e um valor mais reduzido de proteína.

Em termos de deposição lipídica, o conteúdo lipídico do fígado foi maior no tratamento 50L18 do que no 50L12 (63,11±3,98 % vs. 53,01±7,61 %), mas semelhante ao tratamento 44L18 (58,26 ± 10,05 %). Os valores de lípidos musculares não são significativamente diferentes entre os cinco tratamentos, possuindo um valor médio de 2,3 %. O perfil de ácidos gordos (AG) dos diferentes tratamentos não exibe diferenças significativas, possuindo valores médios de AG saturados ≈32%, AG monoinsaturados ≈20%, de AG polinsaturados ≈43% e a relação (n-3)/(n-6) ≈4,2, sendo valores muito bons. Estes resultados, juntamente com os baixos índices aterogénico e trombogénico, prova que a corvina é uma excelente fonte de ómega-3, essenciais param uma condição cardíaca saudável.

Não obstante que a proteína é o ingrediente mais caro na formulação de rações, uma dieta apropriada para a criação de corvina a um nível comercial deverá possuir aproximadamente 50% de conteúdo proteico, e possuir entre 15 a 18% de conteúdo lipídico, sendo estes valores uma boa abordagem para uma dieta ideal que poderia maximizar o crescimento e baixar custos.

Palavras-chave: *Argyrosomus regius*; Rácio proteína:lípido; Crescimento; Perfil lipídico; Ácidos gordos polinsaturados.

ABSTRACT

This study investigates the effects of dietary lipid and protein levels in the growth, feed utilization and body composition of meagre (*Argyrosomus regius*). Triplicate groups of 945 juvenile fish (63,66±2,78 g average weight±SD) were fed for 63 days five isoenergetic diets (21,79±0,17 kJ) containing 44 and 50% of crude protein, and 12, 15 and 18% of crude lipids, named 44L15, 44L18, 50L12, 50L15 and 50L18. Mean values of fishes fed diet 50L18 were significantly higher than all other treatments, regarding final body weight (136,09±1,00 g), specific growth rate (1,21±0,00 %), daily growth index (1,78±0,03 %) and protein efficiency ratio (2,33±0,09), with the %crude protein in the feed to affect the last to parameters. Voluntary feed index of fish was affected by the %crude lipid in the feed, with diet 44L18 to have significantly higher values (1,28±0,11 %) than diets 44L15 and 50L12. Fish fed diet 50L18 had significantly higher values of food conversion ratio (1,04±0,04) than all diets, except diet 50L12. The retention of dry matter and protein had significant differences, with diet 50L18 to have the highest values (27,60±0,98 % and 42,24±2,50 %, respectively), and to also possess the highest daily gain of nitrogen and energy. No significant differences were found in the values of lipid and energy retention, and in the daily gain of lipid. Significant differences were found between the mean lipid deposition on liver, but not on the muscle samples. The fatty acid profile was not significantly different between treatments, and neither were the Atherogenicity and Thrombogenicity indexes. Overall, the results indicated that the best growth performance was observed in fishes fed the 50L18 diet, but with excessive mesenteric fat deposition in the abdominal wall. In conclusion, meagre feeds should have around 50% crude protein and between 15 to 18% crude lipid.

Keywords: *Argyrosomus regius*; Ratio protein:lipid; Growth; Lipid profile; Polyunsaturated fatty acids.

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1 INTRODUCTION

1.1 Aquaculture - present situation and trends:

Aquaculture is one of the most modern types of farming practiced in the World. It started around 2000-1000 B.C., with the Chinese being the first ones to put the knowledge into practice (**Rabanal, 1988**). Nowadays, due to the huge increase in human population, demand for animal products and its derivates is increasing drastically, where aquaculture represents one of the most important forms of supplying the World with food. In 2002, China was the world biggest producer of aquaculture products, in spite of the majority where freshwater products (**Brugère and Ridler, 2004**), followed by India and Indonesia. It's noteworthy that the main producers are found in Asia, and not the developed countries. These countries produce low value fish rather than high value finfish (**Brugère and Ridler, 2004**), which is accordingly with the low income of the population. In 2008, the aquaculture industry accounted for 45,7 % of the world's fish food production for human consumption (**FAO, 2012**), almost half of the world needs, which demonstrates the great growing potential of this industry.

When we talk about the number of species that are produced by aquaculture, the big growth happened within the countries mentioned above, that produced mainly inland water species (**Table 1**):

Table 1.1 – Number of species items with statistics in the FAO capture database. Adapted from **FAO (2012)a**.

	2001	**2010**	**Variation 2001-2010**
	(Number)	***(Number)***	***(Percentage)***
Inland water fish, crustaceans and molluscs	113	190	+68.1
Marine and diadromous fish, crustaceans and molluscs	1 194	1 356	+13.6
Total species items	**1 307**	**1 546**	**+18.3**
Share of inland water species on total species	8.6%	12.3%	

The small increase of marine and diadromous fishes demonstrates the difficulties of growing new species, being necessary to study their complex biology, the feeding habits, reproduction cycles and environmental needs, for artificially grow them in aquaculture systems.

In Europe, the main aquaculture producers are Norway, Spain, France, UK and Italy (**FAO, 2012; Zampogna, 2009**). The marine and diadromous fishes grown are very well known, with extensive studied biology and optimized feeding protocols, such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European eel (*Anguilla*

anguilla), gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) (**Zampogna, 2009**). Due to the relative small diversity of marine fishes grown in Europe, there are proposals of trying to introduce new species in the commercial circuit of aquaculture (**Quéméner, 2002; Roo *et al*., 2010; Chatzifotis *et al*., 2011**), preferably ones that comply with biological (growth, fecundity and growing in captivity), technological (appropriate conversion index, life cycle well known) and market (decline in commercial catches, market breadth, high commercial value and annual availability) favorable characteristics, that, together with a reduced production cost, can compete with the species already cultivated (**Iglesias and Sánchez, 2008; FAO, 2012**).

1.2 The Sciaenidae family:

The Sciaenidae is among the largest families of sonic fishes, including 270 species within 70 genera (**Chao, 1986**). They are sonic fishes, because they emit sounds during the spawning season (primarily), composed of bursts of knocking, drumming or crocking sounds (**Jiménez *et al*., 2005; Ueng *et al*., 2007; Cárdenas, 2010**). With a wide distribution among the temperate and tropical regions of the world (**Jiménez *et al*., 2005**), the great representation of the sciaenids occurs in the Indo-Pacific region, with around 65 species (**Leis and Trnski, 1989**), in the Caribbean, with 17 genera (**Randall, 1983**), and the temperate waters of the Atlantic and Pacific oceans – 2 species are present in the Amazon basin and 5 within the Mediterranean sea (**Fischer, Bauchot and Schneider, 1987**).

According to **Cárdenas (2010) and references there in**, there are seven species being farmed in a commercial and/or experimental level:

- **Japanese meagre** (*Argyrosomus japonicus*) (Temminck and Schlegel, 1843) in Australia (**Silberschneider and Gray, 2008**), South Africa (**Bernatzeder and Britz, 2007; Musson, 2009**), and Taiwan (**Ueng *et al*., 2007**);
- **Corvina drum** (*Cilus gilberti*) (Abbott, 1899) in Chile (**Aburto, 2005; Augsburguer, 2006; Cárdenas *et al*., 2009**);
- **White croaker** (*Micropogonias funieri*) (Demarest, 1823) in Uruguay (**García-Alonso and Vizziano, 2004**);
- **Brown meagre** (*Sciaena umbra*) (Linnaeus, 1758) in Greece (**Chatzifotis *et al*., 2006**) and Turkey (**Claki *et al*., 2006**);
- **Red drum** (*Sciaenops ocellatus*) (Linnaeus, 1766) in China (**Xu *et al*., 2007**), European Union (**Henderson-Arzapalo, 1995; Holt, 2000**), Ecuador (**Rajoy, 2003**), Israel, Martinique (**Dao, 2003; Gardes *et al*., 2000; Soletchnik *et al*., 1989**), Mexico (**García-Ortega and Lazo, 2004; Goffings, 2010**) and Taiwan (**Liao and Chang, 2001**);
- **Shi drum** (*Umbrina cirrosa*) (Linnaeus, 1758) in Cyprus (**Mylonas *et al*., 2000**), Spain (**Arizcun *et al*., 2009**), Greece (**Mylonas *et al*., 2004**), Italy (**Barbaro *et al*., 2002**) and Turkey (**Basaran *et al*., 2009**);
- **Meagre** (*Argyrosomus regius*) (Asso, 1801) in Spain (**Mateos, 2007**), Egypt, France, Italy, Morocco and Turkey (**Jiménez *et al*., 2005**).

1.2.1 Japanese croaker (*Argyrosomus japonicus*) (Temminck and Schlegel, 1843):

A. japonicus is a widely distributed sciaenid fish, being present in the waters of South and Central Australia, from Queensland until Western Australia, where it is known as mulloway, from Hong Kong northwards along the Chinese coast to southern Korea and Japan, and is found in the northern Indian Ocean, from Pakistan to the northwest coast of India (**Griffiths and Heemstra, 1995; Bernatzeder *et al*., 2010**). It is also present in the African southeast coast, from Cape of Good Hope to southern Mozambique, where it is known as dusky kob (**Griffiths and Heemstra, 1995; Jiménez *et al*., 2005**), living until 30 years old and 2 meters of total length (**Jiménez *et al*., 2005**). In Taiwan, fishermen from the Penghu Archipelago culture this species in saltwater net cages, where the maturation occurs at the 5-6 years of age and body length of 70-80 cm, with *A. japonicus* starting to emit sounds at the age of 6 months (**Ueng *et al*., 2007**). Adults spawn in the near-shore marine environment (until depths of approximately 100 m) and early juveniles (>20 mm TL) recruit into estuaries and migrate to the upper reaches where salinity ranges between 0 and 5 ppm. Early juveniles (<150 mm TL) appear to be restricted to the upper reaches, whereas larger juveniles (>150 mm TL) migrate into the middle and lower reaches of estuaries, into the surf zone and eventually out to sea (**Griffiths, 1996; Bernatzeder *et al*., 2010**).

The commercial production started in 1992, in the state of New South Wales (Australia) and since then a large number of fingerlings were successfully produced using pond fertilization techniques (**Jiménez *et al*., 2005**). According to the NSW Fisheries (2003), between 2001 and 2002, were produced 120.000 fingerlings, with a unit price of 0.56 euro (**Jiménez *et al*., 2005**). *A. japonicus* grows at a very good rate in floating cages, around the Sydney area, attaining a size of 45 cm (around 1,1kg) after 26 months of feeding, at environmental temperature, but the commercial size is between 500 and 700 grams (**Jiménez *et al*., 2005**).

1.2.2 Brown meagre (*Sciaena umbra*) (Linnaeus, 1758):

Brown meagre is a demersal species living at depths of 0 to 200 m, with a wide distribution in the Mediterranean Sea, Black Sea and eastern Atlantic Ocean (**Chao, 1986**). It exhibits nocturnal behavior and occupies bottom caves and sea beds covered with vegetation (**Chatzifotis *et al*., 2006; Cakli *et al*., 2006**). Growth is considerably repressed during low temperatures in winter but accelerated from spring until autumn when the water temperature rises. Females grow faster than males. The sexually mature fish spawn from May to July producing pelagic eggs. Feeding is reduced during gonad maturation and brown meagre may use as an energy source hepatic lipid reserves accumulated during the sexual resting period (**Chatzifotis *et al*., 2006; Cakli *et al*., 2006**).

Turkey is the fourth largest producer of farmed fish in the Mediterranean region, having in 2007, a fishery production of 772,471 tons and an aquaculture production of 140,021 tons, being the biggest producer of the 4 Candidate Countries to the EU (**Zampogna, 2009**). Aquaculture of new commercial species, like common dentex, sharpsnout seabream, brown meagre and red sea bream, began in 2000 with few farms and is still a successful activity (**Cakli *et al*., 2006**). The culture of brown meagre, which is being done in small-size operations, is a new pilot activity. For that reason, no studies about the quality of wild of cultured forms of this species exist in Turkey (**Cakli *et al*., 2006**). The species *Sciaena*

umbra has great potential for aquaculture in Turkey and Greece, where the Aegean aquaculture industry is looking for alternative species to culture in sea cages. According to fish sellers, brown meagre obtains high prices because it is one of the preferred fish in Turkey (**Cakli *et al*., 2006**). Knowledge of the nutritional requirements of brown meagre is scarce and the only available information refers to its dietary habits in the wild, but its nutritional requirements are similar with the other *Sciaenidae* species (**Chatzifotis *et al*., 2006; Cakli *et al*., 2006**).

1.2.3 Red drum (*Sciaenops ocellatus)* (Linnaeus, 1766):

The distribution of red drum ranges from Cape Cod in the north-western Atlantic Ocean to Tuxpan, Mexico in the Gulf of Mexico. Red drum or redfish (as this species is also commonly known), usually inhabit coastal and estuarine waters. They have a characteristic red-orange color that can vary from grey to red-bronze and one or more black spots near the base of the caudal fin. Red drum are eurythermal and euryhaline, carnivorous and of considerable commercial value (**Lazo *et al.*, 2010**). Early juveniles feed primarily on bottom-dwelling invertebrates and later stages feed on fish, shrimp and crabs (**Jiménez *et al*., 2005**). Juveniles are found in bays and estuaries until sexually mature, except in their more northerly range, where they move offshore or south in the winter to warmer waters. Red drums are not tolerant to cold temperatures and death results below 10°C (**Lazo *et al.*, 2010**). Sexually mature adults of 3–5 years of age (4–5 kg) migrate to offshore waters and spawning takes place in shallow coastal waters during the evening from August to November. The maximum age documented is 56 years (1250 mm fork length, FL) for males and 52 years (1346 mm FL) for females (**Lazo *et al.*, 2010**).

Its aquaculture started in the 70's, when the adults of this species were induced for the breeding in captivity, with the manipulation of the photoperiod and temperature, and the concomitant development of the larval rearing (**Jiménez *et al*., 2005**), being the first of the *Sciaenidae* to be farmed in a commercial way. This species adapts easily to captivity, laying eggs in laboratory conditions, without the use of hormones, mainly due to good acceptance of artificial feeds and fast grow out, reaching 500 g within 9 months (**Jiménez *et al*., 2005**). The production is made in several states along the Gulf of Mexico and south-eastern Atlantic in the USA, as well as in Taiwan, China, Mexico and several other countries in Latin America (**Lazo *et al*., 2010**).

1.2.4 Meagre (*Argyrosomus regius*) (Asso, 1801):

Meagre, *Argyrosomus regius* (Asso, 1801) is a teleost fish species that belongs to the Sciaenidae family, being one of its largest members (**Chao, 1986**). Can grow more than 180 cm in total length and weight more than 50 kg (**Quéméner, 2002; Prista *et al.*, 2009**). Has a wide distribution, being present in the Mediterranean and Black seas, Atlantic coast of Europe and west coast of Africa, living in inshore or coastal waters, close to the bottom or near the surface (range depths from 15 to 200 m) (**Cabral and Ohmert, 2001; Poli *et al.*, 2003; El-Shebly *et al.*, 2007**). For a few years that it's being proposed as a candidate for the

Mediterranean aquaculture diversification (**Quéméner, 2002; El-Shebly *et al.*, 2007; Chatzifotis *et al.*, 2011**), due to its fast growth, flesh quality and high nutritional value (*regius* for royal quality of flesh) (**Poli *et al.*, 2003; Piccolo *et al*., 2008; Chatzifotis *et al.*, 2011**).

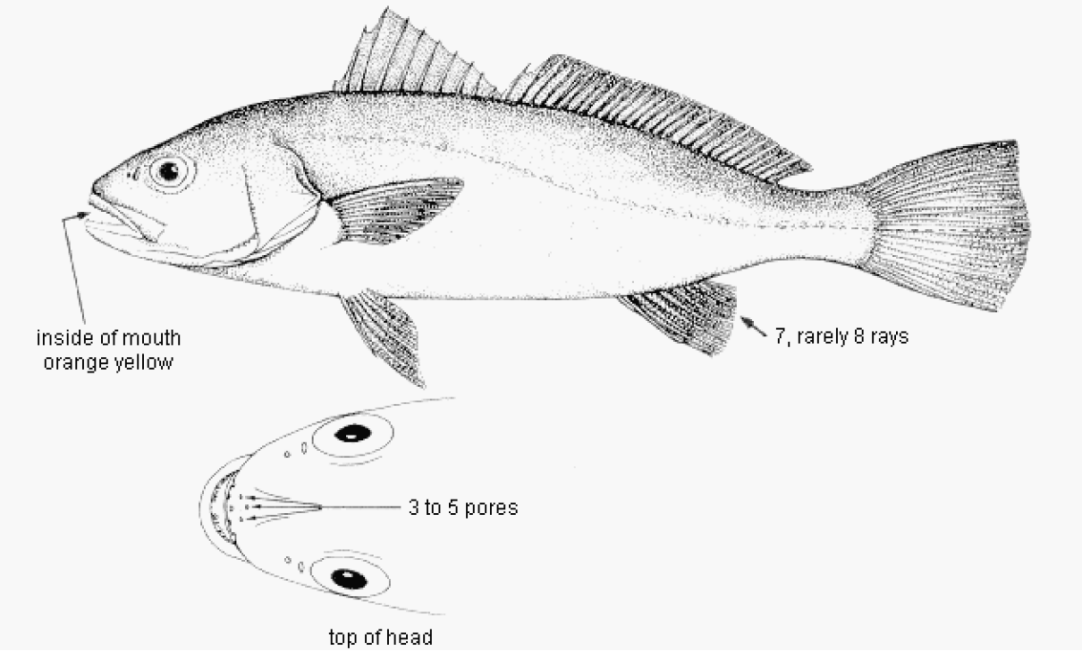


Figure 1.1 – Morphological characteristics of meagre. In **Monfort (2010)**.

In terms of fisheries, the largest ones were recorded in Africa, where Mauritania, Morocco and Egypt are responsible for around 80% of the 10 000 t world annual catch (**Quéméner, 2002; FAO, 2009; Prista *et al.*, 2009**). In Europe, annual values of meagre landings are bellow 500 t, and in Portugal, has a secondary importance (**FAO, 2009; Prista *et al*., 2009**), only being very appreciated in the south region Algarve. Nevertheless, meagre is a large sized fish, can attain high ex-vessel prices and high seasonal availability (**Quéro and Vayne, 1987**; **Prista *et al.*, 2009**), making it a species of great interest for the aquaculture production (**Quéméner, 2002; Jiménez *et al*., 2005; Prista *et al*., 2009**). Adding to those characteristics, it's a specie that tolerates wide ranges of salinity, temperature and can be reared in brackish water ponds (**El-Shebly *et al.*, 2007**), being ideal for the aquaculture industry.

Nowadays, the life cycle is already closed, with many hatcheries spread throughout Europe, and successful grow out in cages or ponds in Mediterranean countries like Greece, France, Italy, Spain and Egypt (**Poli *et al.*, 2003; El-Shebly *et al.*, 2007; Chatzifotis *et al.*, 2011**).

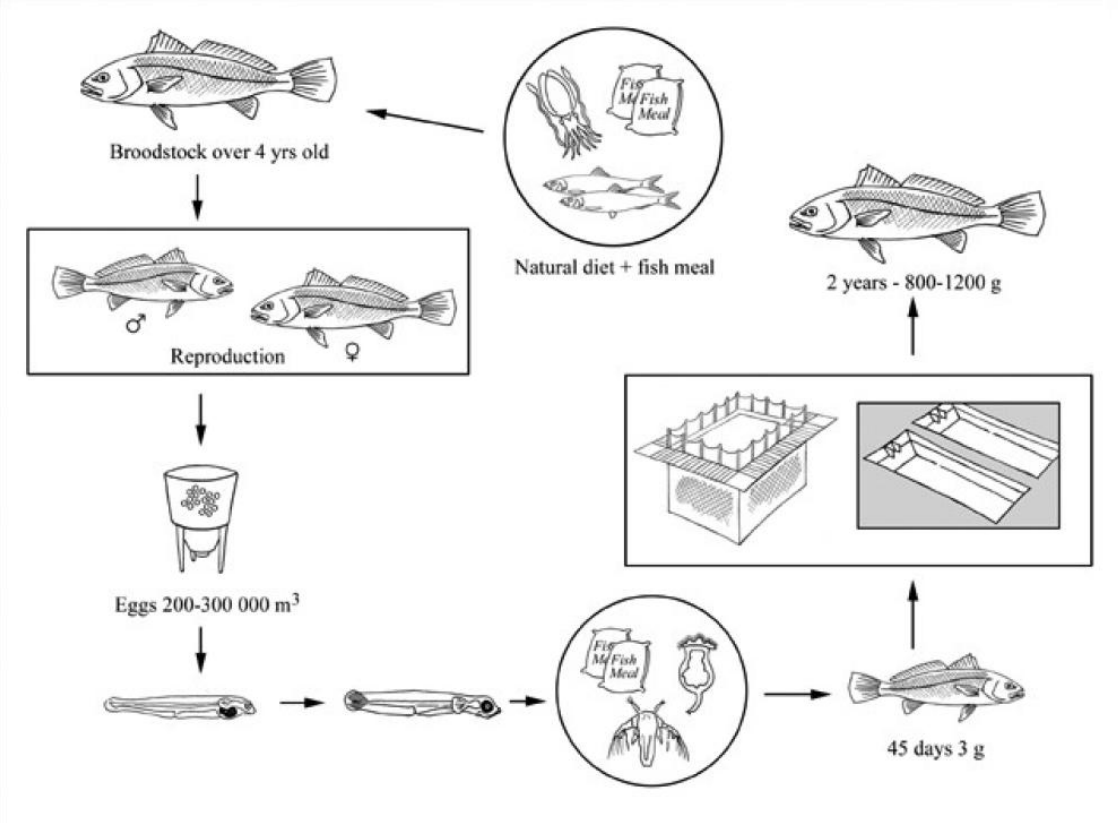


Figure 1.2 – Production cycle of meagre. In **Monfort (2010)**.

In spite of rearing techniques for the different stages are well established, production has not yet reached its full potencial (**Martínez-Llorenz *et al.*, 2011**), mainly because of the feeding protocols, since the techniques used are the same for others species, with well known needs in terms of nutrients.

Meagre is a carnivorous species and in the wild, it feeds on Mysidacea, Decapoda and Teleostei (**Quéro and Vayne, 1987; Cabral and Ohmert, 2001; Jiménez *et al*., 2005; Chatzifotis *et al*., 2011**), but under culture conditions, the feeding protocols used nowadays are based on the pelleted diets used for sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (**Roo *et al*., 2010; Chatzifotis *et al.*, 2011; Martínez-Llorenz *et al.*, 2011**), being inaddequate for this species. Since the necessary dietary requirements for lipids and proteins, that allow to attain the high grow rates for meagre, around **1 kg in less than 1 year** in cages (**Quéméner, 2002; Jiménez *et al*., 2005; Roo *et al*., 2010; Chatzifotis *et al.*, 2011),** are not well know, there is the need of further study to establish the ratio protein:lipid, creating new feeds and feeding protocols (**Roo *et* al., 2010)**, that may be able to fulfill the propose of maximizing growth with the optimum feed, putting a less and less monetary effort on feeds and making possible to reach the full grow potential of this species.

In terms of market value, *Argyrosomus regius* can attain a price from 6 to 10 €/Kg for whole fish (**Jiménez *et al.*, 2005; Monfort, 2010)** and 10-15€/kg for fillets (**Monfort, 2010**), making it a very desirable commercial product (**Hernández *et al*., 2009**). The more versatile a fish can be, in terms of processing and forms of sale, makes it more profitable and worth investing (**Figure 1.3**). Meagre stands for “low fat fish” (from the French *maigre* = slim) (**Monfort, 2010**), and the amount of muscular fat is very low, compared with other

aquaculture species (**Poli *et al*., 2003**), making possible the fillet preservation under refrigeration for longer periods of time, meeting the new lifestyles habits among people: less time to cook and preference for processed products (**Hernández *et al*., 2009**). Many other forms are possible, like smoked fish and sushi, since meagre has a firm white flesh, that keeps it shape after cooking, and with the appropriate marketing, the future looks promising (**Monfort, 2010**).

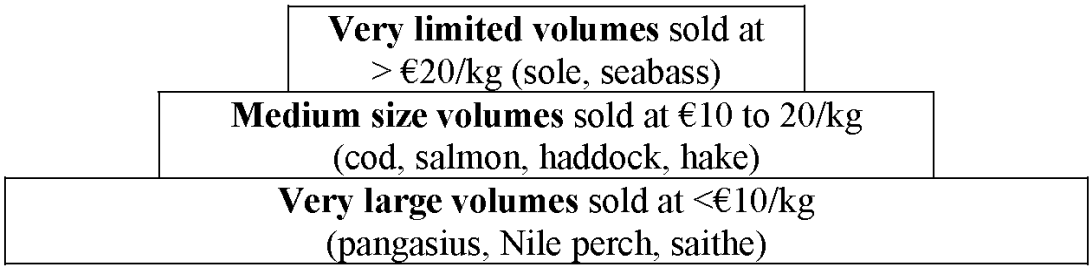


Figure 1.3 – Schematic description of the European retail market for fresh fish portions by consumers' prices categories. In **Monfort (2010)**.

1.3 Nutritional requirements of meagre:

Meagre is categorized as a fish with low fat content (**Poli *et al*., 2003**), with lipid content less than 5%.total weight^{-1} and protein content between 10 and 20%.total weight^{-1} (**Cárdenas, 2010**) (**Table 1.2**), but little information exists about the exact interval of dietary requirements for lipids and proteins that can be used for the formulation of specialized diets for this species. It is established that the level of protein should be superior to 45% and the level of lipids should not exceed 17% (**Cárdenas, 2010**), with many authors researching within or close to this range of values (**Poli *et al*., 2003; Chatzifotis *et al*., 2011; Grigorakis *et al*., 2011; Martínez-Lorenz *et al*., 2011**). **Woolley *et al.* (2010)** refer that the level of protein to be included in feeds should be from 42% to 46%.

Table 1.2 – Muscle fillet contents in fat and protein, from aquaculture reared meagre. Adapted from **Cárdenas (2010)**.

Fish weight (g)	Fat (% total weight)	Protein (% total weight)
195	1,7	20,9
357-385	0,3-0,6	N.D.
665-776	2,4-3,6	20,9-21,1
936-1.503	2,1-2,9	N.D.
2.370	2,49	19,8

These macronutrients provide the required energy, the essential amino acids and the essential fatty acids for growth (**Chatzifotis *et al*., 2011**). Their relative inclusion in diets determines fish growth and the economic performance of aqua feeds, as the proper energy to protein ratio in the diet contributes to the effective utilization of dietary proteins, through the protein sparing effect on fish (**Watanabe, 1982; Chatzifotis *et al*., 2011**). The level of dietary lipids that fish can use is limited, and beyond the point of optimal lipid intake, growth may be retarded, due to reduction in feed consumption, and body fat may increase, affecting the carcass quality (**Chatzifotis *et al*., 2011**). The lipids are mainly supplied through the inclusion of fish oils that contain the necessary amount of fatty acids.

1.3.1 Macronutrients

1.3.1.1 Proteins:

Proteins are the most abundant compounds in living organisms and have fundamental roles in all biological processes. They are organic molecules that contain carbon, hydrogen, oxygen, nitrogen and frequently sulfur. The basic composition of most proteins is very similar, with varying percentages of its components: 50-55% carbon, 20-23% oxygen, 15-18% nitrogen, 6-8% hydrogen, and from 0 to 4% sulfur (**Jobling, 1995**), being composed of up to 20 α-amino acids linked into chains by peptide bonds. The chains are cross-linked by disulfide bridges, hydrogen bonds, and Van der Waals forces (**NRC, 1993**). The amino acid content of proteins, particularly feed proteins, may differ markedly, so the protein nutritional quality derives from the content, proportion and availability of amino acids (**Becker, 2007**).

In the context of animal feeding, protein generally refers to crude protein (CP); that is, N × 6.25, a definition based on the assumption that proteins contain 16% N. The requirement for dietary protein has two components:

1. a need for indispensable amino acids that the fish cannot synthesize either at all or at a rate commensurate with its need for protein deposition or commensurate with the synthesis of a variety of other compounds with metabolic functions and
2. a supply of either dispensable amino acids or sufficient amino nitrogen to enable the fish to synthesize them.

Insofar as synthesis of dispensable amino acids requires expenditure of energy, feeding dietary proteins that most nearly meet the needs of fish for both indispensable and dispensable amino acids, will result in the most efficient growth by the fish (**Thoman *et al*., 1999; Chatzifotis *et al*., 2011**). Dietary protein constitutes one of the primary nutrient costs of the feed and is the initial source of nitrogen waste products entering a culture system. Consequently, optimization of dietary protein levels along with increasing nutrient retention by the fish could reduce nitrogen loading and positively influence production costs (**Mohanta *et al*., 2007**), both from an economical and an environmental perspective. To minimize feed costs, it is important to optimize both dietary protein level and utilization by the fish (**Thoman *et al*., 1999**), and include carbohydrates and lipids.

The amount of protein to be included in a fish diet is influenced by protein to energy ratio (P:E), protein digestibility and amount of non-protein energy in the diet (**NRC, 1993; Mohanta *et al*., 2007**). When insufficient non-protein energy is available in the feed, dietary

protein is delaminated in the body to supply energy rather than being used for tissue growth and protein synthesis. The liver plays a major role in directing the various nutrients to specific organs and tissues to be metabolized for energy (**Li *et al*., 2012**). The same basic metabolic pathways for converting amino acids, carbohydrates and lipid into energy have been observed in fish as in terrestrial animals (**NRC, 1993**). It is preferable for dietary carbohydrates or lipid to be metabolized for energy so that protein (amino acids) can be used for tissue synthesis. To ensure this, there must be a proper balance of dietary protein to energy to optimize fish growth and lean tissue accretion. Energy-to-protein ratios ranging from 8 to 10 kcal of digestible energy/g (DE/g) of protein (33 to 42 kJ/g) are optimal for various fish species (**Gatlin III, 2010**).

Considering this, much research has been conducted to investigate the protein-sparing potential of lipids and carbohydrates in fish diets. In their natural environment, fish have limited access to carbohydrates and are better adapted both at digestive and metabolic levels to utilize protein and lipids than carbohydrates as energy sources (**Li *et al*., 2012**). So, a supplementation of lipids rather than carbohydrates as a non-protein energy source is generally a more effective method to increase dietary energy level because lipids are energy-dense nutrients and are readily metabolized by fish (**NRC, 1993**). In meagre, the protein-sparing effect can be investigated through the optimization of the dietary ratio protein:lipid.

1.3.1.2 Lipids:

Lipids constitute a heterogenic family of compounds with diverse structures. Their common and defining feature is their insolubility in water and solubility in organic solvents (ether, acetone, mixture of chloroform-alcohol). The terms "fats" and "oils" are used to define mixtures of lipids, respectively solids or liquids at room temperature (**Nelson and Cox, 2004**).

The lipids present in teleost fish species may be divided into two major groups: the phospholipids and the triglycerides (**Gunstone *et al*., 2007**). The phospholipids make up the integral structure of the unit membranes in the cells; thus, they are often called structural lipids. The triglycerides are lipids used for storage of energy in fat depots, usually within special fat cells surrounded by a phospholipid membrane and a rather weak collagen network, being formed by the combining of glycerol with three molecules of fatty acids. The triglycerides are often termed depot fat. A few fish have wax esters as part of their depot fats (**Huss, 1995**).

The phospholipids are all contained in membrane structures, including the outer cell membrane, the endoplasmic reticulum and other intracellular tubule systems, as well as membranes of the organelles like mitochondria. In addition to phospholipids, the membranes also contain cholesterol, contributing to the membrane rigidity. In lean fish muscle cholesterol may be found in a quantity of about 6 % of the total lipids (**Huss, 1995; Chatzifotis *et al*., 2010**). The fat cells making up the lipid depots in fatty species are typically located in the subcutaneous tissue, in the belly flap muscle and in the muscles moving the fins and tail (**Huss, 1995**).

Fat depots are also typically found spread throughout the muscle structure. The concentration of fat cells appears to be highest close to the myocommata and in the region

between the light and dark muscle (**Huss, 1995**). The dark muscle contains some triglycerides inside the muscle cells even in lean fish, as this muscle is able to metabolize lipids directly as energy. The corresponding light muscle cells are dependent on glycogen as a source of energy for the anaerobic metabolism (**Huss, 1995; Chatzifotis *et al*., 2010**). To best fulfill the needs in lipids for optimal development and growth, oils derived from fish are used.

1.3.1.2.1 Fish oils:

Fish oils contain a wide range of saturated, monounsaturated, and polyunsaturated acids (**Table 1.3**), but fish fatty acid (FA) composition is generally cited in terms of the major acids only. Such oils are rich in saturated fatty acids (SFAs) (mainly myristic and palmitic), monounsaturated fatty acids (MUFAs) covering the range of hexadecenoic through docosenoic, and omega-3 (n-3) C_{20} and C_{22} polyunsaturated fatty acids (PUFAs) (**Gunstone *et al*., 2007**). These last are very important acids for which fish oils are the largest source, supplying eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. The fish oils vary in the level of combined PUFA's and also in the distribution between EPA and DHA. This is important when selecting a fish oil as a source of one or other of these PUFA for enhancement and when seeking a dietary source rich in one of these acids, but not in the other.

Table 1.3 – Fatty acids identified in fish oils. Adapted from **Gunstone *et al*. (2007)**.

Acid type	Number of carbon atoms
Saturated – straight chain	12, 14-24 odd and even members
Saturated – branched chain	15, 17, 18, 19
Monounsaturated	14, 16, 17, 18, 19, 20, 22, 24
Polyunsaturated	16:2-4, 18:2-4, 20:2-5, 21:5, and 22:3-6

Note: Many of the unsaturated acids occur in several forms.

Fish oils contain materials with valuable dietary and pharmaceutical properties as well as having physical properties that make them useful as moisturizers. Until the 1960s, fish liver oils were used in Europe and the U.S. mainly for their vitamins A and D, but high-quality fish oil is now used as a source of long-chain (n-3) fatty acids (**Simopoulos, 2002; Gunstone *et al*., 2007; Chatzifotis *et al*., 2010**).

1.3.1.2.2 Fatty acids*:*

Fatty acids are simple organic compounds constituted by carbon, hydrogen and oxygen with long hydrocarbon chains of various lengths (4 to 36 carbons long) (**Christie, 1989; Gunstone *et al*., 2007**). Each molecule of fatty acid has in the end of its chain a carboxylic group and in the opposite a non-functional methyl group. Fatty acids are divided

into groups according to chain length, number, position and configuration of their double bonds, and the occurrence of additional functional groups along the chains (**Christie, 1989**). Fatty acids systematic name is derived from the name of its parent hydrocarbon substituting the end *e* with *oic*. For example, the C_{18} saturated fatty acid is called octadecanoic acid since the parent hydrocarbon is octadecane. A C_{18} fatty acid with one double bond is called octadecenoic acid; with two double bounds ("di"), octadecadienoic acid; with three double bonds ("tri"), octadecatrieenoic acid (**Simopoulos, 2002**) (**Table 1.4**).

Table 1.4 - Structure, systematic, trivial, and shorthand names of some common fatty acids, with (n-3) FA marked in **red** and (n-6) FA marked in **blue**. Adapted from **Gunstone *et al*. (2007)**.

Structure	Systematic Name	Trivial Name/ Abbreviation	Shorthand Name	n- or ω
$CH_3(CH_2)_{10}COOH$	Dodecanoic	lauric	12:0	
$CH_3(CH_2)_{12}COOH$	Tetradecanoic	myristic	14:0	
$CH_3(CH_2)_{14}COOH$	Hexadecanoic	palmitic	16:0	
$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	*Z*-9-hexadecenoic	palmitoleic	16:1 9*c*	7
$CH_3(CH_2)_{16}COOH$	Octadecanoic	stearic	18:0	
$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	*Z*-9-octadecenoic	oleic	18:1 9*c*	9
$CH_3(CH_2)_5CH{=}CH(CH_2)_9COOH$	*Z*-11-octadecenoic	*cis*-vaccenic	18:1 11*c*	7
$CH_3(CH_2)_5CH{=}CH(CH_2)_9COOH$	*E*-11-octadecenoic	vaccenic	18:1 11*t*	7
$CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7COOH$	*Z,Z*- 9,12-octadecadienoic	linoleic (LA)	18:2 9*c*,12*c*	6
$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$	*Z,Z,Z*- 9,12,15-octadecatrienoic	α-linolenic (ALA)	18:3 9*c*,12*c*,15*c*	3
$CH_3(CH_2)_3(CH_2CH{=}CH)_3(CH_2)_4COOH$	*Z,Z,Z*- 6, 9,12-octadecatrienoic	γ-linolenic (GLA)	18:3 6*c*,9*c*,12*c*	6
$CH_3(CH_2)_{18}COOH$	eicosanoic[a]	arachidic	20:0	
$CH_3(CH_2)_3(CH_2CH{=}CH)_4(CH_2)_3COOH$	*Z,Z,Z,Z*- 5,8,11,14-eicosatetraenoic[a]	arachidonic (ARA)	20:4 5*c*,8*c*,11*c*,14*c*	6
$CH_3(CH_2CH{=}CH)_5(CH_2)_3COOH$	*Z,Z,Z,Z,Z*- 5,8,11,14,17-eicosapentaenoic[a]	EPA	20:5 5*c*,8*c*,11*c*,14*c*,17*c*	3
$CH_3(CH_2)_{20}COOH$	docosanoic	behenic	22:0	
$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$	*Z*-13-docosenoic	erucic	22:1 13*c*	9
$CH_3(CH_2CH{=}CH)_6(CH_2)_2COOH$	*Z,Z,Z,Z,Z,Z*- 4,7,10,13,16,19-docosahexaenoic	DHA	22:6 4*c*,7*c*, 10*c*,13*c*,16*c*,19*c*	3
$CH_3(CH_2)_{22}COOH$	tetracosanoic	lignoceric	24:0	
$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COOH$	*Z*-15-tetracosenoic	nervonic	24:1 15*c*	9

The n- or “omega” nomenclature is used to describe fatty acids by the general formula X:Yn-z, where X is the carbon chain length, Y is the number of ethylenic/double bonds, and n-z (or ωz) denotes the position of the first double bond relative to the methyl end of the fatty acid. Thus, 16:0 denotes a saturated fatty acid containing 16 carbons and no double bonds (all carbons saturated with hydrogen), and 18:1n-9 (18:1ω9) designates a monounsaturated fatty acid with 18 carbon atoms and a single double bond that is nine carbon atoms from the methyl end. Many freshwater fish can elongate and desaturate 18-carbon linolenic acid with three double bonds to longer chains (20 and 22 carbons) of more highly unsaturated fatty acids (HUFAs) with five or six double bonds. In contrast, most marine fish must have HUFA in the diet (**Gatlin III, 2010**). In the body, HUFAs are components of cell membranes (in the form of phosphoglycerides, or phospholipids), especially in neural tissues of the brain and eye. They also serve as precursors of steroid hormones and the highly active eicosanoids produced from 20-carbon HUFAs. Carbon atoms 2 and 3 are often referred to as α and β, respectively, and the methyl carbon atom at the distal end of the chain is called the ω-carbon. (**Christie, 1989; Gunstone *et al*., 2007**) Fatty acids are named as saturated fatty acids (SFA) when they do not contain double bonds and monounsaturated fatty acids (MUFA) when they contain one double bond. Polyunsaturated fatty acids (PUFA) possess

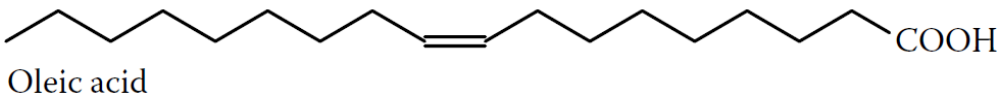
more than one double bond. Many may be present at levels exceeding 10%: SFAs (14:0, 16:0), MUFAs (16:1, 18:1, 20:1, 22:1) and (n-3) PUFAs (20:5, 22:6). Many minor fatty acids are also present (**Gunstone *et al*., 2007**).

1.3.1.2.3 Saturated fatty acids:

SFAs form a homologous series of monocarboxylic acids ($C_nH_{2n+1}COOH$). Naturally occurring saturated acids are mainly of even chain length between C_4 and C_{24}. Odd chain acids are usually minor or trace components of plant and animal lipids (**Christie, 1989**). Palmitic acid (16:0) is the most abundant and widespread natural SFA, present in plants, animals, and microorganisms. Levels of 20 to 30% are common in animal lipids, 10 to 40% in seed oils (**Das, 2006**). Stearic acid (18:0) is also ubiquitous, usually at low levels, but is abundant in cocoa butter (around 34%) and some animal fats, e.g., lard (5 to 24%) and beef tallow (6 to 40%) (**Gunstone *et al*., 2007**).

1.3.1.2.4 Monoenoic fatty acids*:*

Straight-chain, *cis*-monoenoic acids with an even number of carbons are common constituents of many lipids and commodity oils. *Trans*- monoenes are rare components of natural oils and fats. The *cis* (*Z*) double bond is usually inserted by a Δ9-desaturase enzyme into preformed saturated acids; this may be followed by two-carbon chain extension at the carboxyl end. Starting with 16:0, this results in (n-7) monoenes, while desaturation of 18:0 leads to the (n-9) family (**Christie, 1989; Gunstone *et al*., 2007**). The most common monoene is oleic acid (18:1 9*c*) (**Figure 1.4**).



Oleic acid

Figure 1.4 – Oleic acid representation. In **Gunstone *et al*. (2007).**

Oleic acid is found in most plant and animal lipids and is the major fatty acid in olive oil (70 to 75%) and several nut oils, e.g., macadamia, pistachio, pecan, almond, and hazelnut (filbert) contain 50 to over 70%. Palmitoleic acid (16:1 9*c*, (n-7)) is a ubiquitous minor component in animal lipids, somewhat more abundant in fish oils. C_{20} monoenes (9c and 11c) isomers are found in fish oils (**Das, 2006**).

1.3.1.2.5 Methylene-interrupted polyunsaturated acids:

Marine fish contain large amounts of 22:6(n-3) and 20:5(n-3) in the phospholipids of their cellular membranes. Marine fish can neither biosynthesize 22:6(n-3) *de novo* nor from shorter chain precursors such as 18:3(n-3). Therefore, 22:6(n-3) and 20:5(n-3) are essential dietary constituents for marine fish (**Sargent *et al*., 1999**). Most unsaturated fatty acids with two or more double bonds show a characteristic methylene interrupted pattern of unsaturation, with one CH_2 between *cis* double bonds. This pattern results from the operation of a few specific desaturases and chain-elongation enzymes. Plants generally insert double

bonds at the Δ9, Δ12, and Δ15 positions in C_{18} fatty acids, giving (n-9), (n-6), and (n-3) compounds, respectively (**Das, 2006**). Animals can also insert double bonds at the Δ9 position, but not at Δ12 or Δ15; instead, further double bonds are introduced between the carboxyl group and the Δ9 position by Δ5 and Δ6 desaturase enzymes and the chain can then be extended in two carbon units at the carboxyl end of the molecule (**Gunstone *et al*., 2007**). The resulting (n-6) and (n-3) polyenes are shown in **Figure 1.5:**

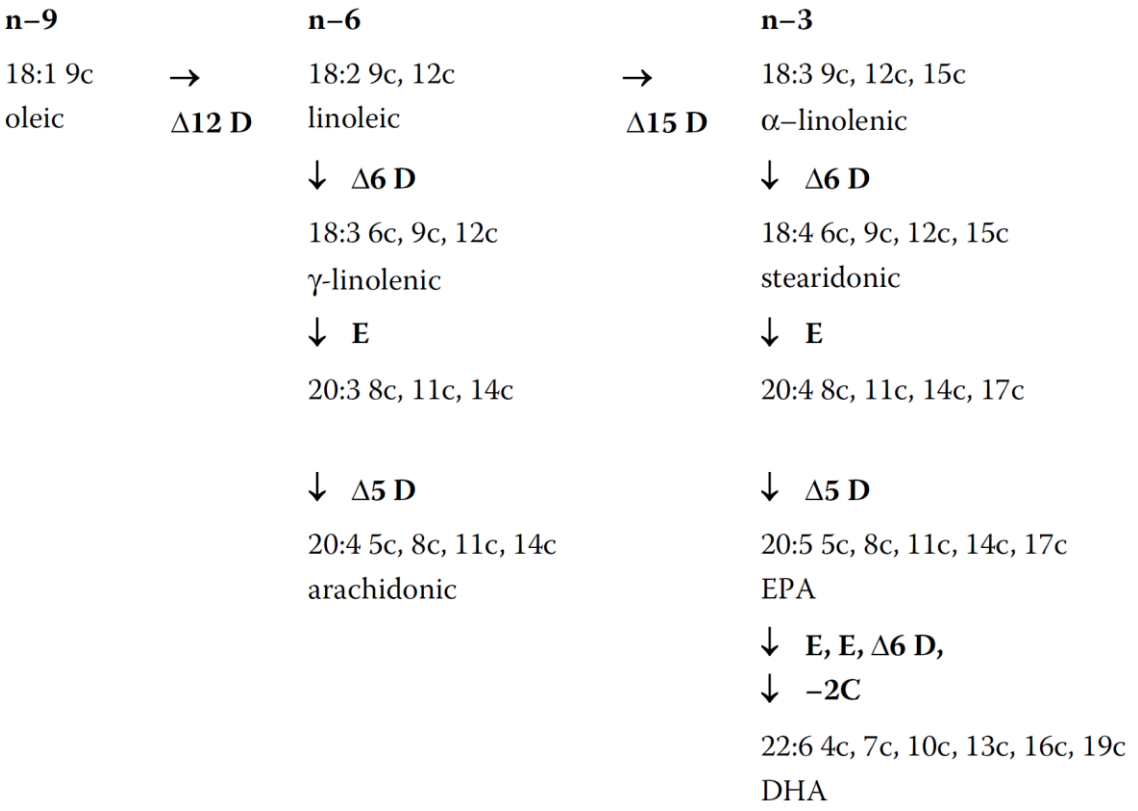


Figure 1.5 - Biosynthesis of (n-6) and (n-3) polyenes (D = desaturase, E = elongase, -2C = two-carbon chain shortening). In **Gunstone *et al*. (2007).**

The step leading to DHA is usually the net result of two elongations, a Δ6 desaturase and subsequent two-carbon chain shortening. Along with a few saturates (mainly 16:0 and 18:0, but also 10:0 to 14:0) and oleic acid, the (n-6) and (n-3) polyenes make up the fatty acids found in most plants, animals, and commodity oils and fats (**Das, 2006**).

Linoleic acid (**LA**, 18:2(n-6)) (**Figure 1.6**) is present in most plant oils and is abundant (>50%) in corn, sunflower, and soybean oils, and exceeds 70% in safflower oil (**Gunstone *et al*., 2007**).

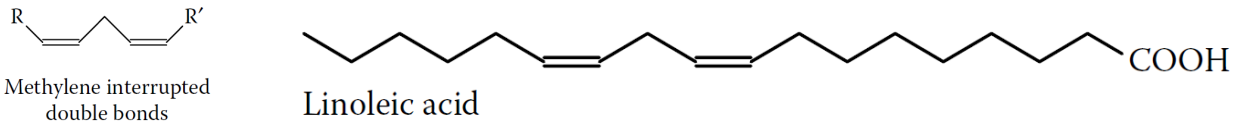


Figure 1.6 – Linoleic acid representation. In **Gunstone *et al*. (2007).**

Arachidonic acid (**ARA**, 20:4(n-6)) (**Figure 1.7**) is present in animal tissues, but do not usually accumulate at significant levels in storage fats, being the precursor of the PG_2 prostaglandin family (**Gunstone *et al*., 2007**).

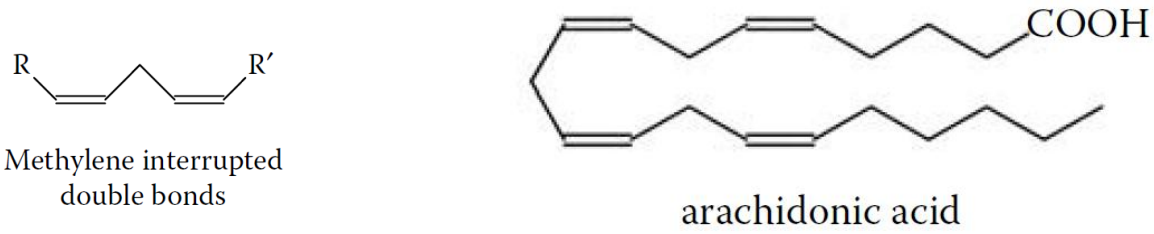


Figure 1.7 – Arachidonic acid representation. In **Gunstone *et al*. (2007).**

Alfa (α) - linolenic acid (**ALA**, 18:3(n-3)) is ubiquitous in plant leaf lipids and is present in several commodity seed oils: 8 to 10% in soybean and canola, >50% in linseed oil, and 65 to 75% of perilla oil (**Gunstone *et al*., 2007**).

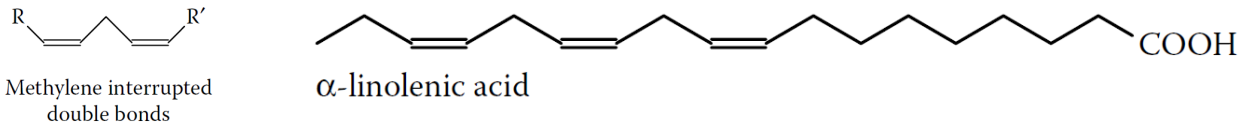


Figure 1.8 – α-linolenic acid representation. In **Gunstone *et al*. (2007).**

The (n-3) long-chain, polyunsaturated fatty acids (LC-PUFA) eicosapentaenoic acid (**EPA**, 20:5 – $CH_3CH_2CH{=}CH(CH_2CH{=}CH)_4CH_2CH_2CH_2COOH$) (**Figure 1.9**), and docosahexaenoic acid (**DHA**, 22:6 – $CH_3CH_2CH{=}CH(CH_2CH{=}CH)_5CH_2CH_2COOH$) (**Figure 1.10**), are important nutritionally and are mainly obtained from oily fish and fish oils where they are present at levels from 5 to 20% (**Das, 2006**). EPA is the precursor of the PG_3 prostaglandin series (**Gunstone *et al*., 2007**).

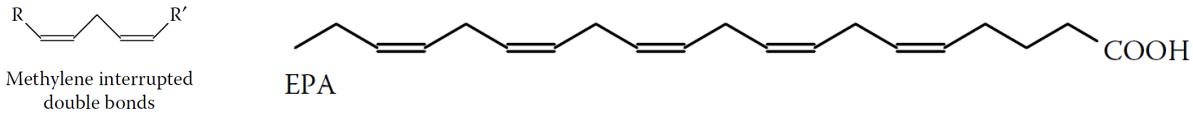


Figure 1.9 – Eicosapentaenoic acid representation. In **Gunstone *et al*. (2007).**

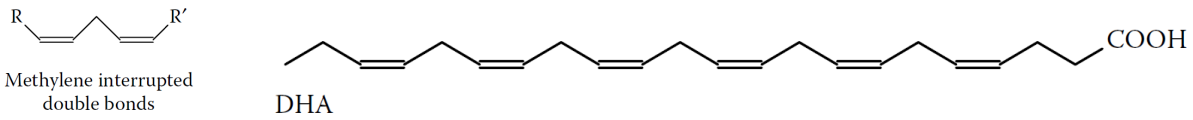


Figure 1.10 – Docosahexaenoic acid representation. In **Gunstone *et al*. (2007).**

Sometimes the total level of (n-3) EPA and DHA is important and on other occasions it is desirable to have high concentrations of only one of them. Sometimes it is desirable to have these acids at higher concentrations than is provided naturally or to enrich one of them at the expense of the other. Alternatively, it may be desired to incorporate one or both of them into an oil rich in medium-chain acids (**Das, 2006**; **Gunstone *et al*., 2007**). The American Heart Association recommends combined intakes of EPA and DHA of 1 g/day for patients with known coronary heart disease (CHD) and 0.5 g/day for individuals without known CHD (**Simopoulos, 2002; Das, 2006**; **Gunstone *et al*., 2007**). The characteristic fatty acids of the fat of fish possess antithrombotic and anti-inflammatory characteristics, justifying a lower prevalence of cardiovascular diseases in fish-eating populations (**Simopoulos, 2002**; **Das, 2006**). In human nutrition, FA such as LA and ALA are regarded as essential since they cannot be synthesized by the organism. In marine fish, these fatty acids constitute only around 2 % of the total lipids, which is a small percentage compared with many vegetable oils (**Simopoulos, 2002**). However, fish oils contain other PUFAs which are "essential" to prevent skin diseases in the same way as LA and ARA (**Das, 2006**). As members of the linolenic acid family (first double bond in the third position, (n-3) counted from the terminal methyl group), they will also have neurological benefits in growing children (**Huss, 1995; Das, 2006**; **Simopoulos, 2002**). **Poli *et al.* (2003)** determined that the flesh of meagre has a fatty acid profile similar to other Mediterranean marine fish (**Cárdenas, 2010**) (**Table 1.5**). Because of these fatty acids contents, meagre possesses anti-atherogenic and antithrombotic properties, measured by indexes. These indexes reflect quantitatively the potential of fats to increase aggression in the vascular endothelium, to produce thrombosis or embolism in subjects sensitive to these conditions. These indexes in meagre are much lower than those posed by ground meat (lamb, beef, pork) (**Poli *et al*., 2003; Piccolo *et al*., 2008**).

Table 1.5 – Fatty acids contents (**%**) of fishes reared in aquaculture. Adapted from **Cárdenas (2010)**.

Species	SFA	MUFA	(n-3) PUFA	(n-6) PUFA	(n-3)/(n-6)	(n-3)+(n-6)
Meagre	**31,1**	**25,7**	**20,9**	**21,4**	**0,9**	**43,2**
Meagre	**26,4**	**24,8**	**17,4**	**14,3**	**1,2**	**31,7**
Meagre	**24,8**	**27,6**	**21,4**	**21,4**	**1,0**	**42,8**
Rubberlip grunt	26,0	27,6	24,6	16,0	1,5	40,6
Gilthead seabream	21,9	37,7	17,4	16,7	1,0	34,1
Redbanded seabream	25,8	22,1	38,3	8,1	4,7	46,4
Red porgy	25,7	31,8	23,6	11,9	2,0	35,6

The main difference between mammalian lipids and fish lipids is that fish lipids include up to 40% of long-chain fatty acids (14-22 carbon atoms) which are highly unsaturated. Mammalian fat will rarely contain more than two double bonds per fatty acid molecule while the depot fats of fish contain several fatty acids with five or six double bonds (**Huss, 1995; Simopoulos, 2002**). The percentage of PUFA with four, five or six double bonds is slightly lower in the PUFA of lipids from freshwater fish (approximately 70%) than in the corresponding lipids from marine fish (approximately 88%) (**Huss, 1995**). However, the composition of the lipids is not completely fixed but can vary with the feed intake and season.

Maintaining high levels of (n-3) PUFA, as well as low levels of (n-6) fatty acids, in farmed fish, is considered desirable to provide a high nutritional value product for human consumption (**Das, 2006**; **Martins *et al*., 2007**).

1.3.1.3 Carbohydrates:

Carnivorous species, such as meagre, are very efficient using dietary protein and lipid for energy but less efficient at using dietary carbohydrates (**Li *et al*., 2012**). The feeds that carnivorous species eat contain little carbohydrate, so they use this nutrient less efficiently. Fish do not have a specific dietary requirement for carbohydrates, but including these compounds in diets is an inexpensive source of energy (**Gatlin III, 2010**). Carbohydrates may serve as precursors for the dispensable amino acids and nucleic acids, which are metabolic intermediates necessary for growth (**NRC, 1993**). The ability of fish to utilize dietary carbohydrates for energy varies considerably; many carnivorous species use them less efficiently than do herbivorous and omnivorous species. Some carbohydrates are deposited in the form of glycogen in tissues such as liver and muscle, where it is a ready source of energy. Some dietary carbohydrates are converted to lipid and deposited in the body for energy (**NRC, 1993; Gatlin III, 2010**). Since fish can't digest complex carbohydrates (cellulose and other fibrous carbohydrates), the amount of crude fiber in fish feeds is usually less than 7% of the diet, to limit the amount of undigested material entering the culture system. Cereal grains serve as inexpensive sources of carbohydrates for warm-water fish, but their use in cold-water fish feeds is limited (**NRC, 1993**).

Soluble carbohydrates such as starch are primary energy reserves found in seeds, tubers and other plant structures. Animal tissues such as liver and muscle contain small concentrations of soluble carbohydrate in the form of glycogen, which is structurally similar to starch (**Gatlin III, 2010**). This glycogen reserve can be rapidly mobilized when the body needs glucose. Prepared feeds for carnivorous fish usually contain less than 20% soluble carbohydrate, while feeds for omnivorous species usually contain 25 to 45%. In addition to being a source of energy, starch in fish feeds also gives pellets integrity and stability and makes them less dense (**NRC, 1993**). Because carbohydrates are the least expensive source of dietary energy, the maximum tolerable concentration should be used with regard to the fish species.

1.3.2 Micronutrients:

1.3.2.1 Vitamins:

Fifteen vitamins are essential for terrestrial animals and for several fish species that have been examined to date (**Table 1.6**).

Table 1.6 – Vitamins and some of their major functions as established in fish. In **Gatlin III (2010)**.

Fat-soluble vitamins	**Function**
vitamin A, retinol	epithelial tissue maintenance, vision
vitamin D, cholecalciferol	bone calcification, parathyroid hormone
vitamin E, tocopherol	biological antioxidant
vitamin K	blood clotting
Water-soluble vitamins	
thiamin, B_1	carbohydrate metabolism
riboflavin, B_2	hydrogen transfer
pyridoxine, B_6	protein metabolism
pantothenic acid	lipid & carbohydrate metabolism
niacin	hydrogen transfer
biotin	carboxylation & decarboxylation
choline	lipotrophic factor, component of cell membranes
folic acid	single-carbon metabolism
cyanocobalamin, B_{12}	red blood cell formation
ascorbic acid, vitamin C	blood clotting, collagen synthesis
inositol	component of cell membranes

Vitamins are organic compounds required in relatively small concentrations to support specific structural or metabolic functions. Vitamins are divided into two groups based on solubility (**NRC, 1993**). Fat-soluble vitamins include vitamin A (retinol), vitamin D (cholecalciferol), vitamin E (α-tocopherol) and vitamin K. These fat-soluble vitamins are metabolized and deposited in association with body lipids, so fish can go for long periods without having these vitamins in the diet before they show signs of deficiency (**Gatlin III, 2010**). Water-soluble vitamins include ascorbic acid (vitamin C), biotin, choline, folic acid, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin and vitamin B12. They are not stored in appreciable amounts in the body, so signs of deficiency usually appear within weeks in young, rapidly growing fish (**Gatlin III, 2010**). Most of these water-soluble vitamins are components of coenzymes that have specific metabolic functions. Vitamin premixes are now available to add to prepared diets so that fish receive adequate levels of each vitamin independent of levels in dietary ingredients. This gives producers a margin of safety for losses associated with processing and storage. The stability of vitamins during feed manufacture and storage has been improved over the years with protective coatings and/or chemical modifications. Therefore, vitamin deficiencies are rarely observed in commercial production (**Gatlin III, 2010**).

1.3.2.2 Minerals:

Minerals consist of inorganic elements the body requires for various purposes. Fish require the same minerals as terrestrial animals for tissue formation, osmoregulation and other metabolic functions. However, dissolved minerals in the water may satisfy some of the metabolic requirements of fish (**Gatlin III, 2010**). Minerals are typically classified as either macro- or microminerals, based on the quantities required in the diet and stored in the body. Macrominerals are calcium, phosphorus, magnesium, chloride, sodium, potassium and sulfur. Dietary deficiencies of most macrominerals have been difficult to produce in fish because of the uptake of waterborne ions by the gills (**Gatlin III, 2010**). However, it is known that phosphorus is the most critical macromineral in fish diets because there is little phosphorus in water. Because excreted phosphorus influences the eutrophication of water, much research has been focused on phosphorus nutrition with the aim of minimizing phosphorus excretion (**NRC, 1993; Li *et al*., 2012**) Phosphorus is a major constituent of hard tissues such as bone and scales and is also present in various biochemicals. Impaired growth and feed efficiency, as well as reduced tissue mineralization and impaired skeletal formation in juvenile fish, are common symptoms when fish have diets deficient in phosphorus (**Gatlin III, 2010**). Chloride, sodium and potassium are important electrolytes involved in osmoregulation and the acid–base balance in the body. These minerals are usually abundant in water and practical feedstuffs. Magnesium is involved in intra- and extracellular homeostasis and in cellular respiration (**Gatlin III, 2010**). It also is abundant in most feedstuffs. The microminerals (also known as trace minerals) include cobalt, chromium, copper, iodine, iron, manganese, selenium and zinc (**Table 1.7**).

Table 1.7 – Trace minerals and some of their functions. In **Gatlin III (2010)**.

Trace mineral	Function
Copper	metalloenzymes
Cobalt	vitamin B_{12}
Chromium	carbohydrate metabolism
Iodine	thyroid hormones
Iron	hemoglobin
Manganese	organic matrix of bone
Molybdenum	xanthine oxidase
Selenium	glutathione peroxidase
Zinc	metalloenzymes

Impaired growth and poor feed efficiency are not readily induced with micro mineral deficiencies, but may occur after an extended period of feeding deficient diets (**Gatlin III, 2010**). Copper, iron, manganese, selenium and zinc are the most important to supplement in diets because practical feedstuffs contain low levels of these microminerals and because

interactions with other dietary components may reduce their bioavailability (**Gatlin III, 2010**). Although it is not usually necessary to supplement practical diets with other microminerals, an inexpensive trace mineral premix can be added to nutritionally complete diets to ensure an adequate trace mineral content.

1.4 OBJECTIVES:

Nowadays, there are no specific feeds for meagre. A 12 weeks feeding trial was undertaken to assess the effect of controlled dietary changes in terms of protein:lipid ratios on the growth performance, nutrient utilization and whole-body composition of juvenile meagre. Moreover, the effect of the various dietary treatments on fat deposition (liver and muscle) and the fillet fatty acid profile was also characterized.

2 MATERIALS AND METHODS

2.1 Experimental diets:

Five isoenergetic (21.92±0.57 MJ/kg mean gross energy) extruded diets were formulated by SPAROS, Lda. (Loulé, Portugal), a commercial feed company, to provide two dietary protein levels of 44% and 50%, and three dietary lipid levels of 12%, 15% and 18%. According to those levels, the feeds were named **44L15**, **44L18**, **50L12**, **50L15** and **50L18**, with first number being %crude protein and second number %crude fat. Ingredients and proximate composition (**Table 2.1**) of the experimental diets were assessed by SPAROS, Lda.

Table 2.1 - Formulation and proximate composition of the experimental diets. By **SPAROS, Lda**.

	44L15	**44L18**	**50L12**	**50L15**	**50L18**
***Ingredients*:**	***%***	***%***	***%***	***%***	***%***
Fishmeal LT (Peruvian)	22,00	22,00	22,00	22,00	22,00
Fishmeal 60/9	15,00	15,00	15,00	15,00	15,00
CPSP 90	3,00	3,00	5,00	5,00	5,00
Squid meal	3,00	3,00	5,00	5,00	5,00
Corn gluten	6,00	6,00	8,50	9,00	10,00
Soybean meal 48	9,00	10,00	13,00	13,00	14,00
Wheat meal	19,00	15,80	15,00	11,80	8,50
Pea bran	10,60	9,80	7,80	7,50	5,50
Fish oil	9,70	12,70	6,00	9,00	12,30
Vitamin & Mineral Premix [1]	1,00	1,00	1,00	1,00	1,00
Dicalcium phosphate (DCP)	1,20	1,20	1,20	1,20	1,20
Binder (Kilseghur)	0,50	0,50	0,50	0,50	0,50
TOTAL	**100,00**	**100,00**	**100,00**	**100,00**	**100,00**

***Proximate composition*:**					
Crude protein	44,51	44,44	50,57	50,46	50,72
Digestible protein	41,39	41,33	47,03	46,92	47,17
Crude fat	15,21	18,17	12,07	15,05	18,38
Fiber	1,53	1,46	1,53	1,45	1,36
Starch	20,41	17,81	16,65	14,36	11,16
Available Phosphorus	1,22	1,21	1,25	1,24	1,24
Gross Energy (MJ/kg)	21,65	22,32	21,17	21,84	22,61

[1] - Minerals (mg/kg diet): Mn (manganese oxyde), 20 mg; I (potassium iodide), 1.5 mg; Cu (copper sulphate), 5 mg; Co (cobalt sulphate), 0.1 mg; Mg (magnesium sulphate), 300 mg; Zn (zinc oxide) 30 mg; Se (sodium selenite) 0.26 mg; Fe (iron sulphate), 56 mg; Ca (calcium carbonate), 80 mg; KCl (potassium chloride), 750 mg; NaCl (sodium chloride), 400 mg. Vitamins (mg/kg diet): Vitamin A (retinyl acetate), 2.75 mg; vitamin D3 (DL-cholecalciferol), 0.04 mg; vitamin K3 (menadione sodium bisulfitete), 10 mg; vitamin B12 (cyanocobalamin), 0.02 mg; vitamin B1 (thiamine hydrochloride), 8 mg; vitamin B2 (riboflavin), 20 mg; vitamin B6 (pyridoxine hydrochloride), 10 mg; folic acid, 6 mg; biotin, 0.7 mg; inositol, 300 mg; nicotinic acid, 70 mg; pantothenic acid, 30 mg.

The major protein sources were from marine-derived ingredients (Fishmeal LT (Peruvian) and Fishmeal 60/9), while fish oil was the main fat source. All feeds, and according to the value of crude protein and/or crude fat, had approximated values for essential amino acids and fatty acids (**Table 2.2**), to avoid any undesired unbalance. All ingredients were finely ground, mixed and extruded (3 mm pellets) by means of pilot-scale twin-screw extruder (CLEXTRAL BC45, France) with a screw diameter of 55.5 mm and temperature ranging 108-114ºC. Upon extrusion, all batches of extruded feeds were dried in a convection oven (LTE OP 750-UF, England) for 4 hours at 50ºC. Following drying, pellets were allowed to cool at room temperature. Subsequently, the fish oil was applied by coating in a vacuum mixer (DINNISEN, PG-10VCLAB, The Netherlands). During the trial, all experimental diets were stored at room temperature, but in a cool and aerated emplacement.

Table 2.2 – Detailed amino acid profile (%) and summarized fatty acid composition (% dry feed) of experimental diets. By **SPAROS, Lda**.

	44L15	**44L18**	**50L12**	**50L15**	**50L18**
***Amino acids*:**	%	%	%	%	%
Arg	2.84	2.84	3.12	3.10	3.09
His	1.14	1.13	1.24	1.23	1.23
Ile	1.88	1.88	2.04	2.03	2.03
Leu	3.37	3.36	3.76	3.77	3.84
Lys	3.02	3.03	3.32	3.30	3.30
Thr	1.77	1.77	1.98	1.97	1.98
Trp	0.45	0.45	0.49	0.49	0.48
Val	2.17	2.16	2.40	2.39	2.40
Met + Cys	1.61	1.60	1.82	1.81	1.82
Phe + Tyr	3.33	3.33	3.69	3.68	3.72
***Fatty acids*:**	%	%	%	%	%
Oleic	1.52	1.86	1.13	1.47	1.84
ARA	0.19	0.22	0.15	0.18	0.22
EPA	1.76	2.22	1.22	1.68	2.18
DHA	1.01	1.25	0.73	0.97	1.24

2.2 Experimental fish and feeding trial:

Manipulations of fish were carried out in compliance with the Guidelines of the European Union Council (EU/63/2010) and Portuguese legislation for the use of laboratory animals. All animal protocols were performed under license of Group-1 from the *Direção-Geral de Veterinária, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas* (Portugal).

The trial was conducted at the Aquaculture Research Station of IPMA in Olhão (Portugal), from September 2011 to December 2011 (63 days), with meagre juveniles bred in captivity at the station. 945 fish (63.66±2.78 g) were randomly distributed into fifteen 1500 L fiberglass tanks (64 fish per tank and each treatment in triplicate) and allowed 1 week for acclimation to experimental conditions before the beginning of the trial. The water was supplied in a flow-through system, with continuous aeration, and physicochemical parameters were measured and registered daily, until the end of the trial (36 $g\ L^{-1}$ salinity, oxygen saturation > 80% and average temperature of 19.8±0.29 °C). Each tank had a stock density of 2.67 Kg/m^3 and received 24 hours of light (dim and natural). The light intensity was measured using the light meter TES 1335, at 11h15, on the first day of the trial, and all tanks had similar values of light intensity. Triplicate groups of fish were hand fed until apparent satiety (*ad libitum*), twice daily (09h00 and 16h00 weekdays, 9h00 and 13h00 weekends), and the feed consumption was recorded daily.

2.3 Sampling methods:

A total of four samplings were made: one in the beginning of the trial, two intermediate, and one final.

In the initial one, all fish were individually weighed and measured, and then returned to the tank of origin. At the beginning of the trial, 15 fish (one from each tank) were sacrificed, individually weighed and stored at − 80 °C for subsequent whole body composition determination (moisture, protein, lipid, energy and ash). For quantification of food intake, and during the week before the intermediate and final samplings, all the feeds that fish wouldn't eat were collected and weighed, so waste could be quantified. The excess food was collected from the bottom of the tank through suction and two hours after the last feeding. The collected feeds would be hydrated, so a correcting factor was applied (**Table 2.3**). To obtain the correcting factor, 5 replicates of 20 g of each feed were immersed in 1 L of water (temperature equal to trial tanks) for 2 hours. Applying the correcting factor to the daily registered values of feed consumption, subtracted by the non ingested feed, would give the daily feed consumption per tank.

Table 2.3 – Hydration factor calculated for each dietary treatment (mean±SD).

Feeds	50L12	50L15	50L18	44L15	44L18
Hydration factor	2.2±2.0	1.92±0.8	1.72±1.0	1.92±0.8	1.92±1.4

After the week where collection of hydrated feeds took place and prior to the intermediate samples, fish were fasted for 24 h, and then, in groups of 10 from each tank, they were counted and weighed individually, and then returned to the tank of origin.

At the end of the trial and after the fast of 24 h, twelve fish from each tank were randomly chosen and anesthetized with 2-phenoxyethanol (0,15 $ml.L^{-1}$). All 12 fish were sacrificed, measured and weighed individually (bench scale Kern & Sohn Gmbh, model ITB 35K1IP, readout 1 g). From those 12 fish, six were stored at -20 °C for whole body composition; from the other six fish, liver and viscera weights were recorded for hepatosomatic and visceral index determination, and frozen at -80 °C, such as muscle fillet samples (area from head to pelvic fin). To avoid the oxidation of the lipid content in the samples of liver, viscera and muscle, and since they will be used for determination of the lipid content and fatty acid composition profile, all were lyophilized during 48h (Heto PowerDry LL3000).

2.4 Analytical methods

2.4.1 Proximate analysis of diets and fish tissues

For determination of the proximate chemical composition of the experimental diets and freeze-dried whole body of fish the following procedures were used: **dry matter** by drying at 105 °C, for 24 h in a P-Selecta 207 oven; **ash** by combustion at 550 °C for 12 h in a muffle furnace; **crude protein** (N×6.25) by the Kjeldahl method after acid digestion; **crude fat** after petroleum ether extraction by the Soxhlet method and a SOXTEC System HT6 extractor; **gross energy** in an adiabatic bomb calorimeter (Werke C2000, IKA, Staufen, Germany). Amino acids profiles of diets were obtained after hydrolysis in 6 M HCL at 108 °C over 24 h in nitrogen-flushed glass vials. A Waters Pico-Tag reversed phase HPLC system, using norleucine as an internal standard, was used. The resulting chromatograms were analyzed with Breeze software (Waters, USA). Tryptophan was not analyzed since it is destroyed by acid hydrolysis, while glutamine and asparagine are converted to glutamate and aspartate, respectively, during acid hydrolysis.

2.4.2 Growth performance

Biological evaluation of feed ingredients and finished feeds involves feeding fish and analyzing some aspect of fish performance and/or diet digestibility. Therefore, several indices should be calculated, to assess the performance of feeds, their retention in the carcass, and the values of nutrients that fish gain through the trial. At the end of the trial, to assess the performance of feeds, indices were calculated – Specific Growth Rate (**SGR**), Feed Conversion Ratio (**FCR**), Protein Efficiency Ratio (**PER**), and Voluntary Feed Index (**VFI**) – retention, in which the deposition of a nutrient in the carcass over a short time is measured, and gain, was calculated for protein, fat and energy.

Accurate prediction of the growth potential of a fish stock, under given husbandry conditions, is an inevitable prerequisite to estimate the energy or feed requirement (e.g.,

weekly ration). The formula most commonly used for fish growth rate expression is the instantaneous growth rate, known as the **Specific Growth Rate**, which is based on the natural logarithm of body weight, and can be used to compare growth on a daily basis:

$$\mathbf{SGR} = \frac{\mathbf{[\ln(FBW) - \ln(IBW)]}}{\mathbf{Days}} \times \mathbf{100}$$

In this formula, **FBW** is the final mean body weight (g); **IBW**, the initial mean body weight (g); and **D**, the number of days. The SGR has been widely used by most biologists to describe the growth rate of fish, being dependent on the IBW, with comparisons of growth rates among groups made with similar IBW.

One of the most important ratios is the **Feeding Conversion Ratio**, being the quantity of feed fed divided by fish weight gain over a specific time period, with values typically ranging from 1.5 to 0.8 in intensive fish culture (0.8 is a better ratio). The true FCR includes wasted feed and mortalities. The ratio, usually expressed as a true ratio (i.e. 1**:**1.5) is often quoted as a "rate" (1.5). FCRs of less than 1**:**1 are possible with commercial diets, as the pellet being fed is a "dry" diet, and a high percentage of weight gained by the fish, is water trapped in the tissues and cells. Feed conversion ratios with commercial "dry" diets are typically in the region of 1**:**0.8 to 1**:**1.5. Ratios with wet diets are higher than this, and can be as high as 1**:**10. FCR varies according to several factors, including the nutritional and physical quality of the aquafeed; environmental variants, such as temperature; the intensity of production (and therefore the availability or not of 'natural' feed); and other factors, including genetics (**New and Wijkström, 2002**)**.** The basic principle is to feed the fish right up to the point of fullness. If they are totally satisfied, fish will not be in stress and will provide quality food for human consumption. This requires that the aquiculture technician has the ability to accurately judge the amount of food to be provided in each situation. With the technological development of aquaculture, many devices have been developed to assist in determining the amount of food to provide for obtaining optimal feeding regimens. The formula for FCR is defined as:

$$\mathbf{FCR} = \frac{\mathbf{Dry\ Feed\ Supplied}}{\mathbf{Fish\ Wet\ Weight\ Gained}}$$

A useful method to compare protein sources in a single experiment is the **Protein Efficiency Ratio**, a measure of the weight gain per unit protein fed. There is a standard PER method (**AOAC, 1998**) involving rats. In this method, proteins are compared at a suboptimal dietary level. This ratio can tell the balance efficiency between protein and energy (protein sparing effect), and can measure the deposition of lipids by the same sparing effect. The PER is calculated as follows:

$$\mathbf{PER} = \frac{\mathbf{Wet\ Weight\ Gain}}{\mathbf{(Crude\ Protein\ Intake \times \%Feed\ Protein)}}$$

In this ratio, the crude protein intake in related to the percentage of protein present in the feed, to accurately calculate the efficiency of retention of the protein present in the feed.

Finally, a measure of feed intake, by weight, and by day, is calculated through **Voluntary Feed Intake**, standing as a percentage, is calculated by:

$$\mathbf{VFI} = \frac{\mathbf{Crude\ Feed\ Intake}}{\mathbf{(IBW \times Days)}} \times \mathbf{100}$$

This formula gives a notion of the percentage of feed that fish consume to increase their weight, on a daily basis. Temperature, diseases, light, and many others factors, that unbalance the respective comfort-zone, will have an increase or reduction on feed intake, varying VFI. If fish get satiated with feeds that have a good efficiency (and they are fully on their comfort-zone), their VFI would be lower, since they need to intake less feed to grow and maintain their balance.

The retention of specific nutrients or energy in the whole body of fish over a specific time period is a useful way of evaluating the availability and balance of amino acids and the availability of some essential elements and other nutrients as well. Based on data from feed intake and whole-body composition of fish, nutrient and energy retention (expressed as percentage of intake) as well as daily nutrient gain were calculated as follow:

$$\mathbf{Retention} = \mathbf{100} \times \frac{\mathbf{(FBW \times F.\ carcass\ nutrient\ cont.\ -\ IBW \times I.\ carcass\ nutrient\ cont.)}}{\mathbf{Nutrient\ Intake}}$$

The livers were removed and weighed and expressed as percentage of body weight and used to calculate hepatosomatic (HSI) index:

$$\mathbf{HSI\ (\%)} = \frac{\mathbf{Liver\ Weight}}{\mathbf{Body\ Weight}} \times \mathbf{100}$$

The HSI provides an indication on status of energy reserve in an animal. In a poor environment, fish usually have a smaller liver (with less energy reserved in the liver).

2.4.3 Total lipids

Lipid content of liver and muscle tissue was determined using two different protocols, to assess differences: a modified **Folch *et al*. (1957)** method and petroleum ether extraction by the Soxhlet method using a SOXTEC System HT6 extractor (AOAC, 1998). The lipid percentage was then calculated by gravimetric analysis. It includes the weight of all lipid components, including glycerol, soluble in the solvent system.

2.4.3.1 Fatty acid profile

Fatty acid methyl esters (FAMEs) were prepared according to **Lepage and Roy (1986)**. The FAMEs preparation was carried out using 0.1 g of freeze dried material and 5 ml of acetyl chloride:methanol mixture (1:19, v/v). The esterification was carried out at 80°C

over an one hour period. The organic phase was collected, filtered and dried over anhydrous sodium sulfate. Solvent was removed under nitrogen and the FAMEs dissolved in 0.1 ml of n-heptane.

The analysis was performed in a gas chromatograph Varian CP-3800 (Walnut Creek, CA, USA) equipped with an auto-sampler and fitted with a flame ionization detector at 250°C. Separation was done in a polyethylene glycol capillary column DB-WAX with 30 m in length, 0.25 mm i.d., and 0.25 µm film thicknesses from J&B Scientific (USA). The column was subjected to a temperature program starting at 180°C for 5 minutes, heating at 4°/minutes for 10 minutes, and held up at 220°C for 25 minutes. The temperature of the injector (split ratio 100:1) was kept constant at 250°C during the 40 minutes analysis. The quantification was done using the area of the C21:0 internal standard. All analytical determinations were done in triplicate. Fatty acid methyl esters were identified by comparison with the retention times of known mixtures of standards (Supelco, FAME 37 and PUFA 3).

To measure the propensity of meagre to influence the incidence of coronary heart disease (CHD), two indices, the Index of Atherogenicity (IA), as a measure of fat deposition in arteries, and the Index of Thrombogenicity (IT), that measures the potential of fatty acids to provoke thrombosis, were calculated according to **Ulbricht and Southgate (1991)** equations:

$$IA = \frac{12{:}0 + 4(14{:}0) + 16{:}0}{PUFA(n\text{-}3) + PUFA(n\text{-}6) + MUFA}$$

$$IT = \frac{14{:}0 + 16{:}0 + 18{:}0}{0{,}5(MUFA) + 0{,}5PUFA(n\text{-}6) + 3PUFA(n\text{-}3) + (n\text{-}3/n\text{-}6)}$$

Two ratios related with fatty acid content were also calculated: (n-3)/(n-6) and PUFA/SFA, in order to allow comparisons with the United Kingdom Department of Health recommendations (**UKDH, 1994**).

2.5 Statistical analysis

Statistical analyses followed methods outlined by **Zar (1999)**. All data is expressed as (mean±SD). Data concerning dietary treatments were analyzed by a one-way ANOVA with STATISTICA 11.0 software package (StatSoft Inc., Tulsa, OK, USA). Since the experimental design was not orthogonal, a one-way ANOVA was used to identify differences between the 2 dietary protein levels and the 3 dietary lipid levels, at p <0,05, and their weight in the data. When these tests showed significance, pair-wise comparisons (Tukey's HSD) were used to determine the differences between means. If the data did not meet the assumptions of an ANOVA (Levene's test for homogeneity of variance and Shapiro-Wilk's test for normality), a non-parametric one-way Kruskal-Wallis ANOVA was used to compare means at p <0,05.

3 RESULTS

3.1 Proximate analysis of diets

Survival rate of the fish in different experimental tanks varied between 95,2 and 100% with no significant difference (p <0,05) in fish mortality among different diet groups.

The proximate chemical composition of the five experimental diets is presented in (**Graph 3.1**). Comparing these values (obtained in the beginning of the trial) with the values assessed in the formulation of the feeds (**Table 2.1**), there are no differences, in terms of nutrients. The amounts of protein and lipids remain the same, the diets are isoenergetic, and the amount of phosphorus is equal.

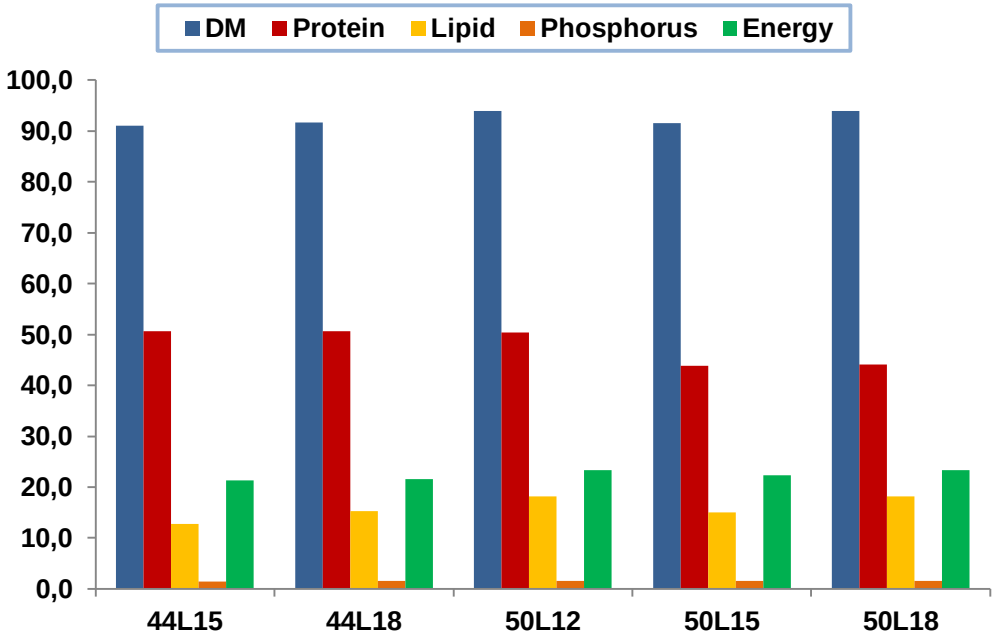


Figure 3.1 – Composition of the five different treatments. Mean values of dry matter (DM), protein, lipid and phosphorus are in %, and energy values are in MJ/kg.

3.2 Proximate analysis of whole body composition

The values obtained, on a dry matter basis, between the tissues of fish fed the different feeds and the initial ones, differ significantly (**Figure 3.2**). Treatment **50L12** had the highest value of protein (69,9±3,11 %) and the lowest values of energy (21,8±0,71 MJ/kg) and lipid (16,8±2,86 %), being the lipid value lower than the initial one (19,6±0,14 %). Treatment **55L18** had the lowest value of ash (14,5±0,99 %), compared with the highest value (15,0±0.85 %) of treatment **50L15**. Treatment **44L18** presented the highest values for lipid (22,6±2,91 %) and DM (28,8±0,65 %).

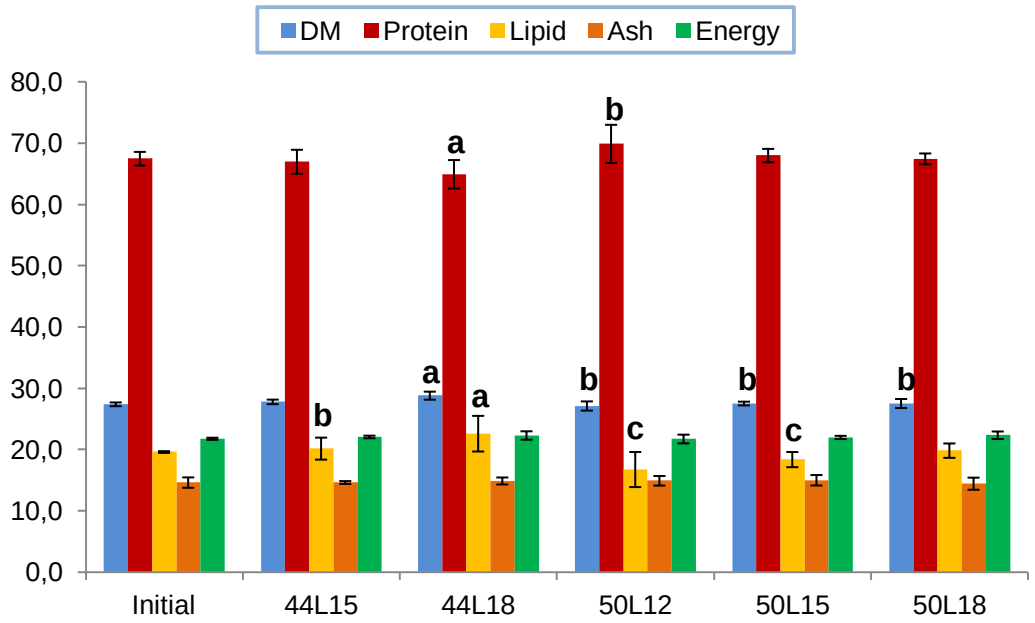


Figure 3.2 – Dry matter composition of fish sampled initialy and after the 63 days trial, per treatment. Bars with different subscript letters differ significantly (p <0,05) and standard deviations are represented by vertical bars.

On a fresh matter basis, and comparing the tissues of fish fed the different feeds and the initial ones (without treatment), treatment **50L12** had higher values of moisture (72,8±0,75 %) and protein (19,0±0,94 %), and the lowest values of lipid (4,6±0,90 %) and energy (5,9±0,35 MJ/kg). Treatment **55L18** had the lowest value of ash (4,0±0,18 %), compared with the highest value (4,3±0,19 %) of treatment **44L18**. Apart from these differences, and as presented in **Figure 3.3**, all treatments had similar values.

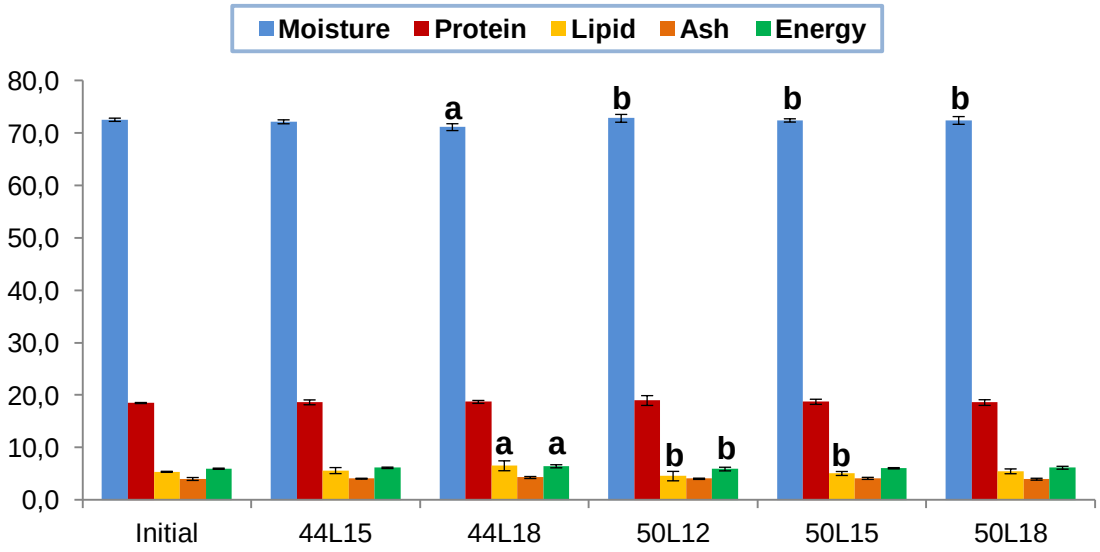


Figure 3.3 – Fresh matter composition of fish sampled initialy and after the 63 days trial, per treatment. Bars with different subscript letters differ significantly (p <0,05) and standard deviations are represented by vertical bars.

Regarding the one-way ANOVA analysis of the whole-body composition (**Table 3.1**), DM and moisture mean values of fishes fed diet **44L18** were significantly different (p <0,05) than fishes fed diets **50L12**, **50L15** and **50L18**; dry matter protein means were higher (p <0,05) in feed **50L12** than **44L18**; dry matter lipid means of feed **44L18** were significantly higher (p <0,05) than **44L15**, **50L12** and **50L15**, with the last two being lower than feed **44L15**; fresh matter lipid means of feed **44L18** were significantly higher (p <0,05) than feeds **50L12** and **50L15**; fresh matter energy means of feed **44L18** were significantly higher (p <0,05) than feed **50L12**; between means of dry matter ash and energy, and fresh matter ash, no differences (p >0,05) were found. The dietary lipid level is highly significant (p <0,01) in the means of dry matter protein, dry matter lipid, and fresh matter lipid, being significant (p <0,05) in the fresh matter energy. Dietary protein is highly significant (p <0,01) in the means of dry matter DM, dry matter lipid, fresh matter moisture and fresh matter lipid, being significant (p <0,05) in the means of dry matter protein.

Table 3.1 – Whole-body composition of meagre fed the various experimental diets over 63 days.
Values represented as (mean±SD); (n=32).
Within a row, means with different letters differ significantly (P <0,05) upon a one-way ANOVA of all treatments. Absence of superscript indicates no significant difference between treatsments.
Diets were further analysed by means of a one-way ANOVA, with levels of lipid (L) and protein (P) as variables.*****P <0,05;******P <0,01;NS – Not Significant.

	Dietary treatments						ANOVA	
	Initial	44L15	44L18	50L12	50L15	50L18	L	P
Dry matter (%)								
DM	27,4±0,31	27,8±0,37	28,8±0,65**a**	27,2±0,75**b**	27,6±0,30**b**	27,6±0,74**b**	NS	**
Protein	67,5±1,12	67,0±1,95	65,0±2,32**a**	69,9±3,11**b**	68,0±1,09	67,5±0,89	**	*
Lipid	19,6±0,14	20,2±1,79**b**	22,6±2,91**a**	16,9±2,86**c**	18,4±1,24**c**	19,9±1,17	**	**
Ash	14,7±0,85	14,7±0,23	14,9±0,57	14,9±0,78	15,0±0,85	14,5±0,99	NS	NS
Energy (MJ/kg)	21,8±0,17	22,1±0,21	22,3±0,69	21,8±0,71	22,0±0,26	22,4±0,62	NS	NS
Fresh matter (%)								
Moisture	72,6±0,31	72,2±0,37	71,2±0,65**a**	72,9±0,75**b**	72,5±0,30**b**	72,4±0,74**b**	NS	**
Protein	18,5±0,09	18,6±0,46	18,7±0,28	19,0±0,94	18,8±0,49	18,6±0,54	NS	NS
Lipid	5,4±0,10	5,6±0,58	6,5±0,94**a**	4,6±0,90**b**	5,1±0,38**b**	5,5±0,46	**	**
Ash	4,0±0,28	4,1±0,08	4,3±0,19	4,1±0,12	4,1±0,20	4,0±0,18	NS	NS
Energy (MJ/kg)	6,0±0,12	6,2±0,14	6,4±0,29**a**	5,9±0,35**b**	6,1±0,12	6,2±0,27	*	NS

3.3 Growth performance

The different performances of the five experimental feeds are represented in **Figure 3.3**. The feed **50L18** had the highest values of SGR – specific growth rate (1,21±0,00 %) and PER – protein efficiency ratio (2,33±0,09), and presented the lowest value for FCR – food conversion ratio (1,04±0,04). The experimental feed **44L18** had the highest values of FCR (1,45±0,11) and VFI – voluntary feed index (1,28±0,11 %), and the lowest value of PER

(1,49±0,12). Feed **44L15** had the lowest values for SGR (0,88±0,09 %), and feed **50L12** had the lowest value of VFI (0,99±0,07 %).

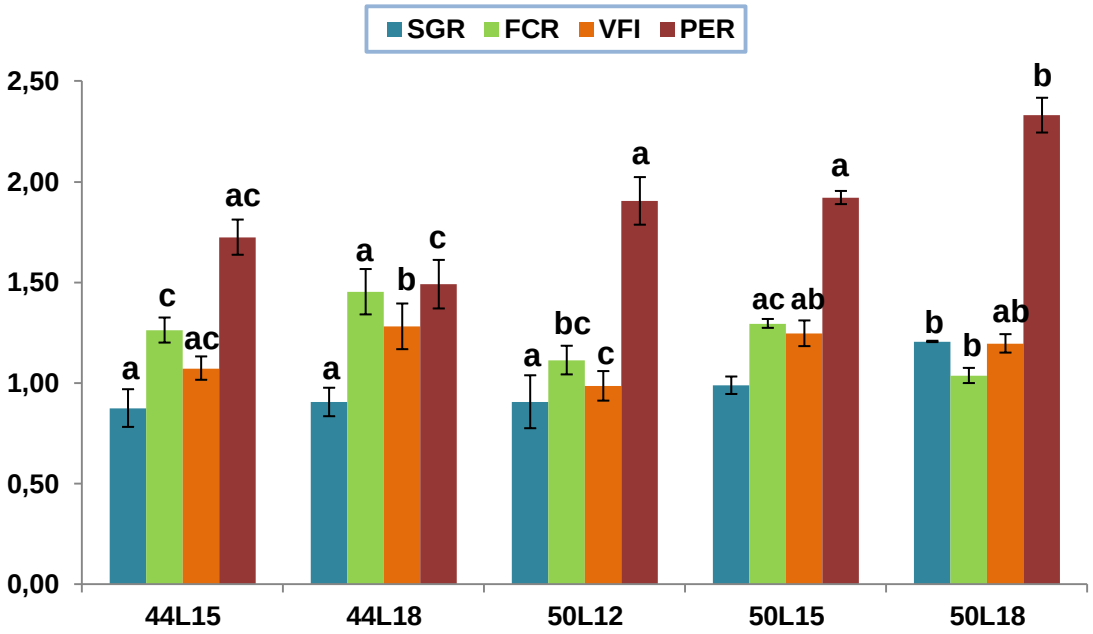


Figure 3.3 – Growth performance of the five different feeds. Bars with different subscript letters differ significantly (p <0,05) and standard deviations are represented by vertical bars.

The mean values of FBW – final mean body weight – and feed intake are represented in **Figure 3.4**, since the range of values is much higher than the other parameters, represented in **Figure 3.3**. Feed **50L18** had the highest values for FBW (136,09±1,00 g) and feed intake (4670,33±138,66 g), while feed **44L15** had the lowest value for FBW (110,66±5,89 g) and feed **50L12** had the lowest value of feed intake (3626,00±591,55 g).

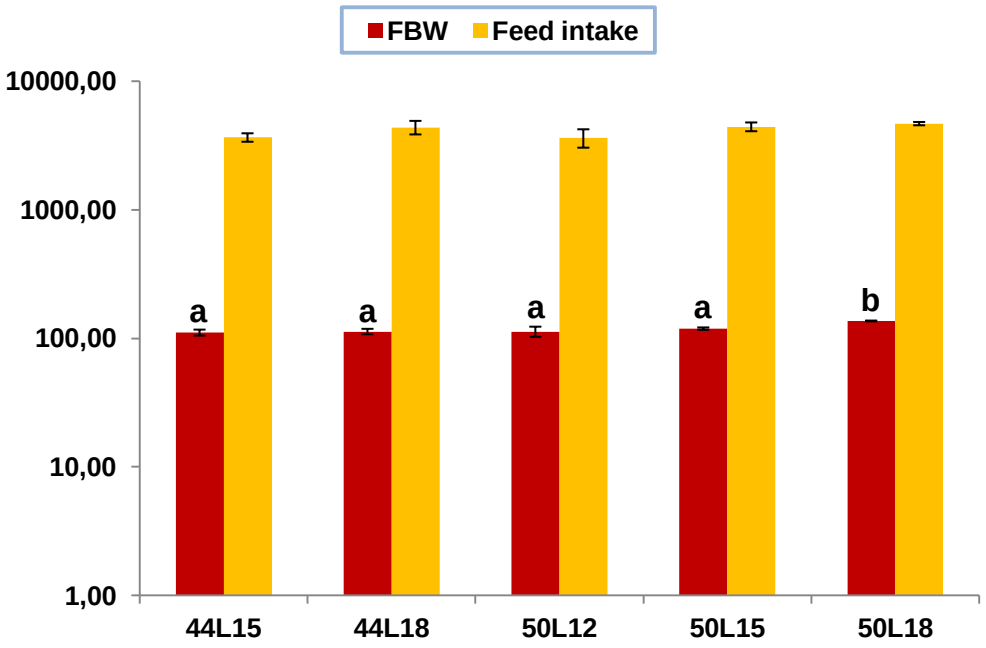


Figure 3.4 – Bars with different subscript letters differ significantly (p <0,05) and standard deviations are represented by vertical bars. The scale is $\log_{10}(Y)$.

Regarding the one-way ANOVA analysis of the growth performance (**Table 3.2**), FBW means of feed **50L18** were significantly higher (p <0,05) than all the other feeds; VFI means of feed **44L18** were significantly different (p <0,05) from feeds **44L15** and **50L12**, and feeds **50L15** and **50L18** significantly higher (p <0,05) than **50L12**; SGR means of feed **50L18** were significantly higher (p <0,05) than **44L15**, **44L18** and **50L15**; FCR means of feed **44L18** were significantly higher (p <0,05) than all feeds, except **50L15**, that was significantly different (p <0,05) from feed **50L18**, which had the lowest mean value; PER means of feed **50L18** were significantly higher (p <0,05) than all feeds, and feeds **50L12** and **50L15** were significantly higher (p <0,05) than feed **44L18**; feed intake means possess no significant differences (p >0,05) between treatments. The dietary lipid level is highly significant (p <0,01) between the means of VFI and significantly different (p <0,05) between the means of feed intake. Dietary protein is highly significant (p <0,01) in the means of FCR and PER.

Table 3.2 – Growth performance of meagre fed the various experimental diets over 63 days. Values are (mean±SD); (n=15).
Within a row, means with different letters differ significantly (P <0,05) upon a one-way ANOVA of all treatments. Absence of superscript indicates no significant difference between treatsments.
Diets were further analysed by means of a one-way ANOVA, with levels of lipid (L) and protein (P) as variables.*P <0,05;**P <0,01;NS – Not Significant.
IBW – Initial mean body weight (g).
FBW – Final mean body weight (g).
VFI – Daily voluntary feed intake.
SGR – Specific growth rate.
FCR – Food convertion ratio.
PER – Protein efficiency ratio.

	Dietary treatments					ANOVA	
	44L15	44L18	50L12	50L15	50L18	L	P
Growth performance							
FBW (g)	110,66±5.89**a**	112,75±5.46**a**	112,66±10.19**a**	118,70±2.52**a**	136,09±1.00**b**	NS	NS
VFI, %IBW/d	1,07±0,06**ac**	1,28±0,11**b**	0,98±0,07**c**	1,25±0,06**ab**	1,20±0,05**ab**	**	NS
Feed intake	3650,33±275,98	4375,67±528,36	3626,00±591,55	4420,33±346,66	4670,33±138,66	*	NS
SGR (%)	0,87±0,09**a**	0,90±0,07**a**	0,91±0,13**a**	0,99±0,04	1,21±0,00**b**	NS	NS
FCR	1,26±0,06**c**	1,45±0,11**a**	1,11±0,07**bc**	1,30±0,02**ac**	1,04±0,04**b**	NS	**
PER	1,72±0,09**ac**	1,49±0,12**c**	1,90±0,12**a**	1,92±0,03**a**	2,33±0,09**b**	NS	**

In **Figure 3.5** are represented the mean Hepatosomatic Indexes (HSI) of each diet. Taking the mean global HSI (2,04±0,36), fishes fed diet **44L18** had the highest HSI (2,36±0,33) and fishes fed diet **50L12** had the lowest HSI (1,55±0,28). No significant differences were found between diets.

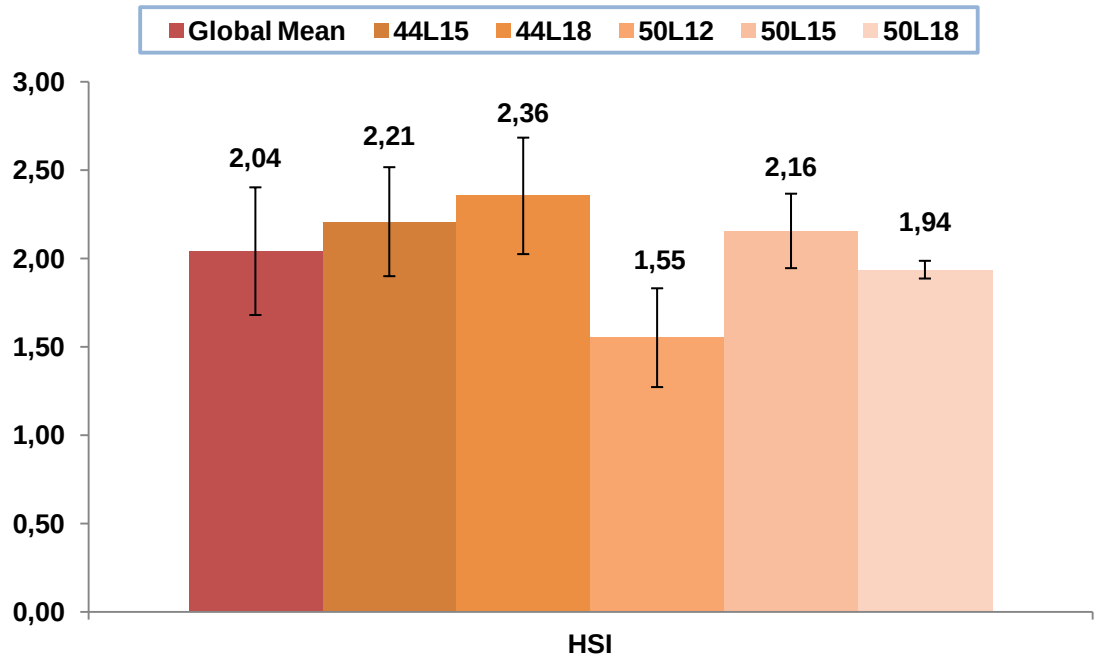


Figure 3.5 – Comparison between the global means (n=6) of HSI with mean values from the five diferent diets. Standard deviations are represented by vertical bars.

3.4 Nutrient retention and gain

In **Figure 3.6** is represented the nutrient and energy retention, during the 63 days trial. Feed **50L18** had the highest retention values of DM (27,60±0,98 %), protein (42,24±2,50 %) and energy (26,98±0,47 kJ), and feed **44L15** had the highest value of lipid retention (39,59±9,69 %). Feed **44L18** had the lowest mean retentions for DM (22,51±1,62 %) and protein (27,57±3,94 %), feed **50L12** had the lowest mean retention for lipid (17,65±13,01 %) and feed **50L15** had the lowest mean retention of energy (22,61±0,09 kJ).

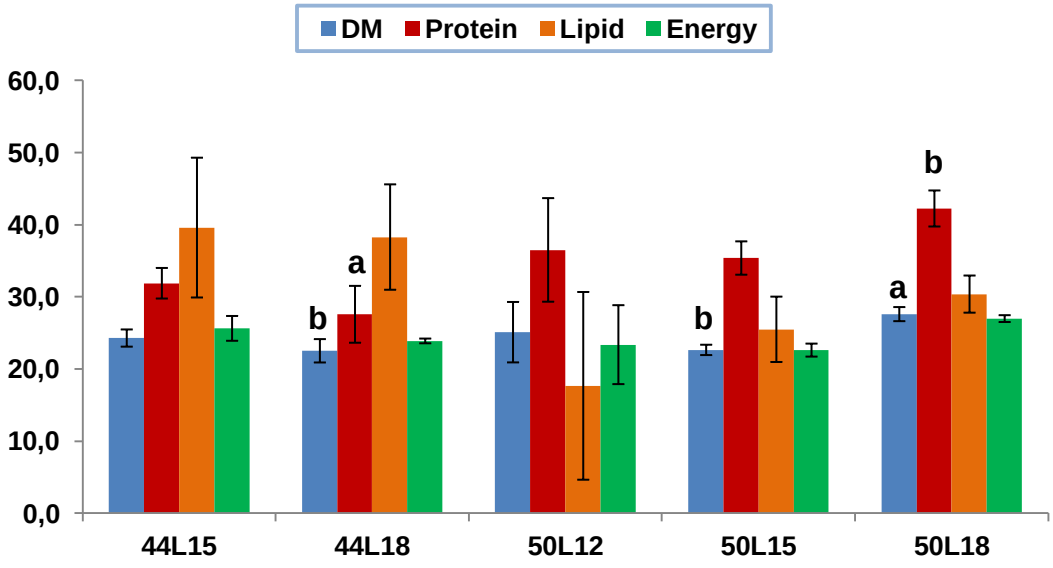


Figure 3.6 – Nutrient and energy retention of the five different feeds. Bars with different subscript letters differ significantly (p <0,05) and standard deviations are represented by vertical bars.

Figure 3.7 and **3.8** represent the daily nutrient gain - nitrogen (N) and lipid - and daily gain in energy. Feed **50L18** had the highest gain in N (334,56±8,26 mg) and energy (0,71±0,04 kJ), and feed **44L18** had the highest gain in lipid (0,69±0,17 g). The lowest means were recorded in feed **44L15** for N (251,87±19,52 mg) and feed **50L12** for lipid (0,31±0,25 g) and energy (0,51±0,16 kJ).

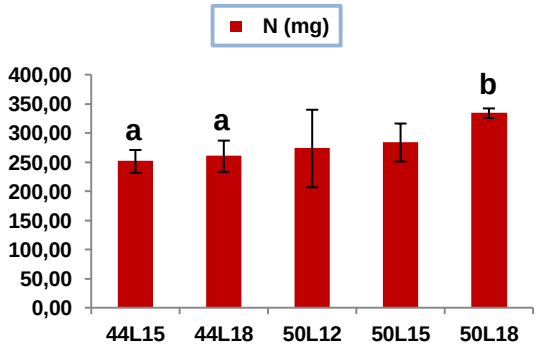


Figure 3.7 – Mean (n=3) daily N gain (mg/kg), per treatment. Bars with different subscript letters differ significantly (p <0,05) and standard deviations are represented by vertical bars.

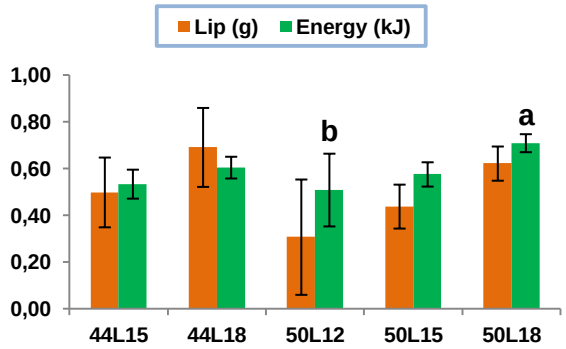


Figure 3.8 – Mean (n=3) daily lipid gain (g/kg) and daily energy gain (kJ/kg), per treatment. Bars with different subscript letters differ significantly (p <0,05) and standard deviations are represented by vertical bars.

In **Table 3.3** are represented the one-way ANOVA's regarding mean retention and mean daily gain of nutrients and energy of the experimental feeds. Protein retention of feed **50L18** was significantly higher (p <0,05) than feed **44L18**; nitrogen gain of feed **50L18** was significantly higher (p <0,05) than feed **44L15** and **44L18**, and energy retention mean values were significantly higher (p <0,05) in feed **50L18** than **50L12**; retention of DM, lipid, energy, and lipid daily gain means possess no significant differences (p >0,05) between treatments. The dietary lipid level is highly significant (p <0,01) between the means of lipid retention, and significantly different (p <0,05) between the means of lipid daily gain. Dietary protein is highly significant (p <0,01) in the means of protein retention and lipid retention.

Table 3.3 – Nutrient and energy retention and daily gain in meagre fed the various experimental diets over 63 days.
Values are (mean±SD); (n=15).
Within a row, means with different letters differ significantly (P <0,05) upon a one-way ANOVA of all treatments. Absence of superscript indicates no significant difference between treatsments.
Diets were further analyzed by means of a one-way ANOVA, with levels of lipid (L) and protein (P) as variables.*P <0,05;**P <0,01;NS – Not Significant.

	Dietary treatments					ANOVA	
	44L15	44L18	50L12	50L15	50L18	L	P
Retention (% intake)							
DM	24,28±1,19	22,51±1,62**b**	25,09±4,19	22,64±0,72**b**	27,60±0,98**a**	NS	NS
Protein	31,88±2,12	27,57±3,94**a**	36,50±7,18	35,37±2,31	42,24±2,50**b**	NS	**
Lipid	39,59±9,69	38,28±7,31	17,65±13,01	25,49±4,53	30,37±2,57	**	**
Energy	25,62±1,72	23,88±0,33	23,37±5,47	22,61±0,90	26,98±0,47	NS	NS

Daily gain (g, mg or kJ/kg/day)							
N (mg)	251,87±19,52**a**	260,67±26,87**a**	273,94±66,56	284,15±32,69	334,56±8,26**b**	NS	NS
Lipid (g)	0,50±0,15	0,69±0,17	0,31±0,25	0,44±0,09	0,62±0,07	*	NS
Energy (kJ)	0,53±0,06	0,61±0,05	0,51±0,16**b**	0,58±0,05	0,71±0,04**a**	NS	NS

3.5 Total lipids

The assessment of total lipids and its deposition was made through the analysis of the liver, digestive tract and muscle. Digestive tract was analyzed according to **Folch *et al.* (1957)** protocol, and liver and muscle samples were analyzed through Soxhlet method. As stated in **Figure 3.9**, fishes fed the treatment **50L18** had the highest mean percentages for lipid deposition in liver (63,11±3,98 %), digestive tract (23,64±3,00 %) and muscle (2,86±1,43 %). Fishes fed diet **44L18** had the lowest mean percentage of muscle lipid deposition (1,96±0,57 %), the ones fed with **50L12** had the lowest mean percentage of lipid deposition on liver (53,01±7,61 %), while the ones fed with treatment **50L15** had the lowest mean percentage of lipid deposition on the digestive tract (17,56±1,73 %).

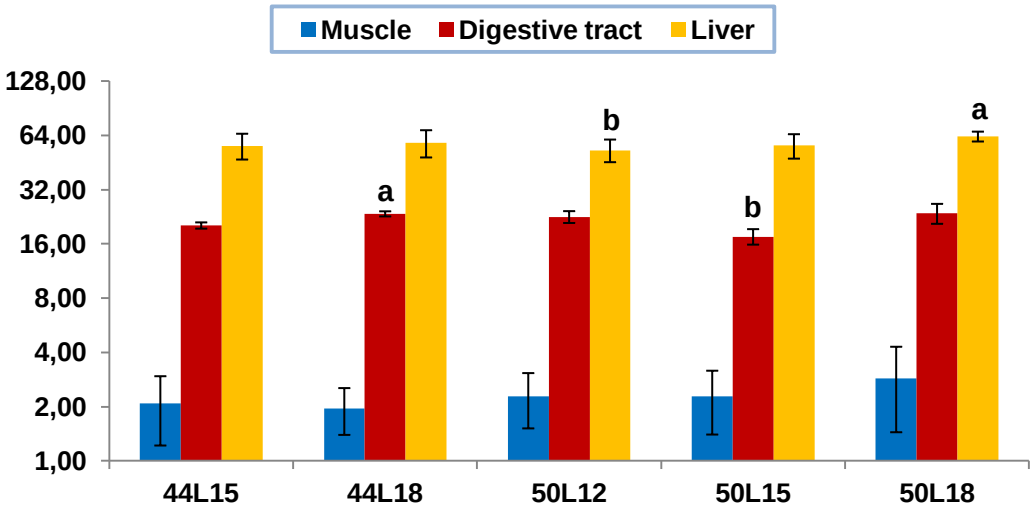


Figure 3.9 – Lipid deposition in liver, digestive tract, and muscle. Bars with different subscript letters differ significantly (p <0,05) and standard deviations are represented by vertical bars. Vertical axis is represented as $\log_2(Y)$.

In **Table 3.4** are represented the mean percentages of lipid deposition in the liver, digestive tract and muscle, per treatment. Since the data didn't meet the requirements for the one-way ANOVA (normality and homoscedasticity), a Kruskal-Wallis ANOVA was performed. Regarding the mean values of liver lipids, fishes fed the experimental feed **50L18** had significantly higher (p <0,05) mean values than **50L12**, and fishes fed the treatment **44L18** had significantly higher (p <0,05) mean values of digestive tract lipids than fishes fed the diet **50L15**; there were no significant differences (p >0,05) between the mean values of muscle lipids. The dietary lipid level is significantly different (p <0,05) between the means of liver lipids and digestive tract lipids, per treatment. Dietary protein does not statistically affect (p >0,05) the lipid deposition.

Table 3.4 – Mean values of lipid deposition in liver, digestive tract and muscle, per treatment, over 63 days.
Values are (mean±sd); (liver and muscle: n=9; digestive tract: n=18).
Within a row, means with different letters differ significantly (*P* <0,05) upon Kruskal-Wallis ANOVA of all treatments. Absence of superscript indicates no significant difference between treatsments.
Diets were further analysed by means of Kruslal-Wallis ANOVA, with levels of lipid (L) and protein (P) as variables.***P*** <0,05; NS – Not Significant.

	Dietary treatments					ANOVA	
	44L15	44L18	50L12	50L15	50L18	L	P
Lipid deposition							
Liver (Soxhlet)	56,13±9,18	58,26±10,05	53,01±7,61**b**	56,22±8,71	63,11±3,98**a**	*	NS
Digestive tract (Folch)	20,22±0,81	23,46±0,77**a**	22,57±1,71	17,56±1,73**b**	23,64±3,00	*	NS
Muscle (Soxhlet)	2,08±0,86	1,96±0,57	2,29±0,78	2,28±0,88	2,86±1,43	NS	NS

3.6 Fatty acid profile

The fatty acid profile of meagre fed the five different diets is showed in **Table 3.5**. There were no statistically differences (*p* <0,05) between the fatty acid profiles. Fishes fed treatment **50L15** had a significant content of muscle SFA (34,49±5,83 %), due to high content in palmitic (16:0 - 23,47±4,78 %) and stearic (18:0 - 8,07±0,70 %) acids, having the lowest contents in MUFA (18,52±1,27 %), DHA [22:6(n-3) - 20,47±4,88 %] and PUFA (41,39±5,60 %). Muscle samples from fishes fed treatment **50L12** had the lowest content in SFA (30,35±3,00 %) and EPA [20:5(n-3) - 7,69±0,48 %], but had the highest content in MUFA (21,58±2,96 %), mainly due to the high percentage of oleic acid (18:1(n-9) - 11,95±0,82 %) and the presence of 22:1(n-11) (1,01±0,23 %). Treatment **50L18** presented the highest percentage of muscle EPA (20:5(n-3) - 9,12±0,23 %), while treatment **44L15** had the highest percentage of muscle DHA [22:6(n-3) - 23,04±4,61 %] and PUFA (44,26±6,22 %).

The highest and lowest content of muscle (n-3) fatty acids was, respectively, found in treatment **44L15** (36,14±6,20 %) and treatment **44L18** (32,75±1,30 %), mainly due to differences in DHA content. The sum of (n-6) fatty acids showed that fishes fed the diet **50L12** had the highest content (9,50±0,57 %), while fishes fed diet **44L15** had the lowest content (7,26±0,07 %) in (n-6) fatty acids. The highest (n-3)/(n-6) fatty acids ratio was recorded in fishes fed diet **44L15** (4,98±0,01 %), and the lowest in diet **50L12** (3,52±0,01 %), which also presented the highest PUFA/SFA ratio recorded (1,45±0,23 %).

Table 3.5 – Fatty acid methyl ester profile of meagre fed each different treatment. Values are in percentage (%) of total muscle fatty acids (mean±sd; n=3); N.D. – not detected.

Fatty acid (%)	44L15	44L18	50L12	50L15	50L18
11:0	N.D.	0,01±0,02	0,01±0,02	N.D.	0,04±0,02
14:0	1,82±0,41	2,23±0,22	1,89±0,74	1,91±0,31	2,83±1,22
15-isobr	0,02±0,04	0,05±0,04	0,03±0,05	0,04±0,03	0,09±0,03
15:0	0,33±0,07	0,34±0,03	0,32±0,06	0,36±0,04	0,39±0,11
16:0	21,13±5,30	20,19±1,55	19,64±2,58	23,47±4,78	19,89±2,50
17:0-isobr	0,29±0,03	0,34±0,35	0,40±0,27	0,18±0,17	0,26±0,05
17:0	0,38±0,03	0,38±0,03	0,37±0,03	0,44±0,05	0,48±0,06
18:0	7,96±0,45	7,17±0,75	7,23±0,86	8,07±0,70	6,88±0,77
19:0-isobr	0,05±0,05	0,33±0,49	N.D.	0,03±0,05	0,12±0,02
19:0	0,23±0,01	0,07±0,12	0,23±0,02	N.D.	0,24±0,02
20:0	0,23±0,05	0,78±0,93	0,23±0,02	N.D.	N.D.
Σ SFA	**32,45±6,21**	**31,88±2,49**	**30,35±3,00**	**34,49±5,83**	**31,21±3,14**
16:1(n-9)+(n-7)	3,28±0,47	3,62±0,66	3,42±1,51	3,39±0,25	4,52±1,68
17:1	0,16±0,04	0,38±0,45	0,61±0,17	0,16±0,01	0,13±0,02
18:1(n-9)	10,68±0,51	11,15±1,20	11,95±0,82	10,84±0,80	11,06±0,54
18:1(n-7)	3,27±0,56	3,80±1,07	2,76±0,35	2,96±0,22	3,03±0,30
18:1(n-5)	0,03±0,05	N.D.	0,13±0,15	0,10±0,11	0,06±0,05
20:1(n-9)	1,12±0,18	0,74±0,65	1,71±0,20	1,08±0,28	1,63±0,23
20:1(n-7)	N.D.	0,04±0,06	N.D.	N.D.	0,11±0,03
22:1(n-11)	N.D.	N.D.	1,01±0,23	N.D.	0,05±0,09
22:1(n-9)	N.D.	N.D.	N.D.	N.D.	0,57±0,99
Σ MUFA	**18,54±1,39**	**19,72±1,21**	**21,58±2,96**	**18,52±1,27**	**21,17±3,43**
16:2(n-4)	0,51±0,11	0,59±0,04	0,53±0,05	0,61±0,07	0,57±0,14
16:3(n-4)	0,29±0,10	0,25±0,05	0,37±0,02	0,38±0,21	0,38±0,28
16:3(n-3)	0,60±0,25	0,52±0,29	0,49±0,33	0,81±0,13	N.D.
16:4(n-3)	0,36±0,20	0,33±0,21	0,24±0,20	0,48±0,03	0,26±0,18
18:2(n-6)	4,62±0,24	5,85±1,44	6,79±0,50	5,31±0,38	5,63±0,31
18:3(n-6)	0,16±0,03	0,15±0,14	0,13±0,04	0,09±0,08	0,15±0,01
18:3(n-4)	0,07±0,12	0,07±0,06	0,17±0,09	N.D.	0,13±0,06
18:3(n-3)	0,51±0,09	0,62±0,19	0,64±0,12	0,52±0,08	0,66±0,10
18:4(n-3)	0,75±0,13	0,81±0,07	0,69±0,19	0,72±0,11	1,07±0,25
20:2(n-6)	N.D.	0,18±0,02	0,22±0,03	0,05±0,09	0,20±0,01
20:4(n-6)	1,89±0,09	1,64±0,14	1,76±0,35	1,79±0,08	1,59±0,29
20:4(n-3)	0,34±0,29	0,46±0,02	0,45±0,04	0,26±0,22	0,53±0,02
EPA 20:5(n-3)	**8,82±0,93**	**8,14±0,17**	**7,69±0,48**	**8,04±1,04**	**9,12±0,23**
21:5(n-3)	N.D.	N.D.	N.D.	N.D.	0,25±0,03
22:4(n-6)	N.D.	N.D.	N.D.	N.D.	0,43±0,74
22:5(n-6)	0,60±0,15	0,33±0,29	0,60±0,18	0,31±0,27	0,29±0,26
22:5(n-3)	1,73±0,40	1,06±0,92	1,80±0,23	1,56±0,56	1,29±1,14
DHA 22:6(n-3)	**23,04±4,61**	**20,82±0,49**	**21,46±5,07**	**20,47±4,88**	**20,94±5,71**
Σ PUFA	**44,26±6,22**	**41,81±2,31**	**44,02±5,42**	**41,39±5,60**	**43,50±5,34**
Σ(n-3)	**36,14±6,20**	**32,75±1,30**	**33,46±5,29**	**32,85±6,24**	**33,87±6,58**
Σ(n-6)	**7,26±0,07**	**8,15±1,18**	**9,50±0,57**	**7,55±0,58**	**8,30±0,81**
Σ(n-3)/Σ(n-6)	**4,98±0,01**	**4,02±0,01**	**3,52±0,01**	**4,35±0,01**	**4,08±0,01**
PUFA/SFA	**1,36±0,29**	**1,31±0,34**	**1,45±0,23**	**1,20±0,48**	**1,39±0,26**

In **Figure 3.10** are represented the mean global percentages of identified SFA, MUFA and PUFA, through gas chromatography, of the meagre muscle samples, per experimental treatment. These values correspond to the ones presented in **Table 3.5**.

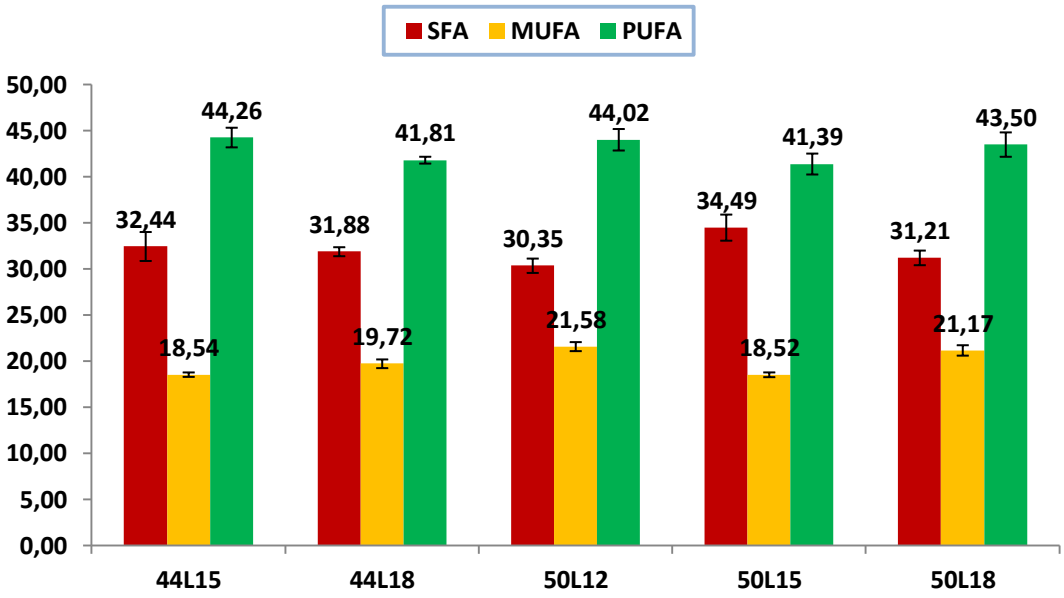


Figure 3.10 – Total SFA (saturated fatty acid), MUFA (monounsaturated fatty acid) and PUFA (polyunsaturated fatty acid) of the muscle samples, per experimental feed. Mean values (n=3) are represented in percentage (%) and standard deviations are represented by vertical bars.

In **Figure 3.11** are represented the mean values of concentration in mg/100g, of total SFA, MUFA and PUFA, together with the concentration of total (n-3) and (n-6) fatty acids, total EPA and DHA. These values were assessed at the end of the trial, through gas chromatography, of the feed samples (Fs) and muscle samples (Ms) from each treatment.

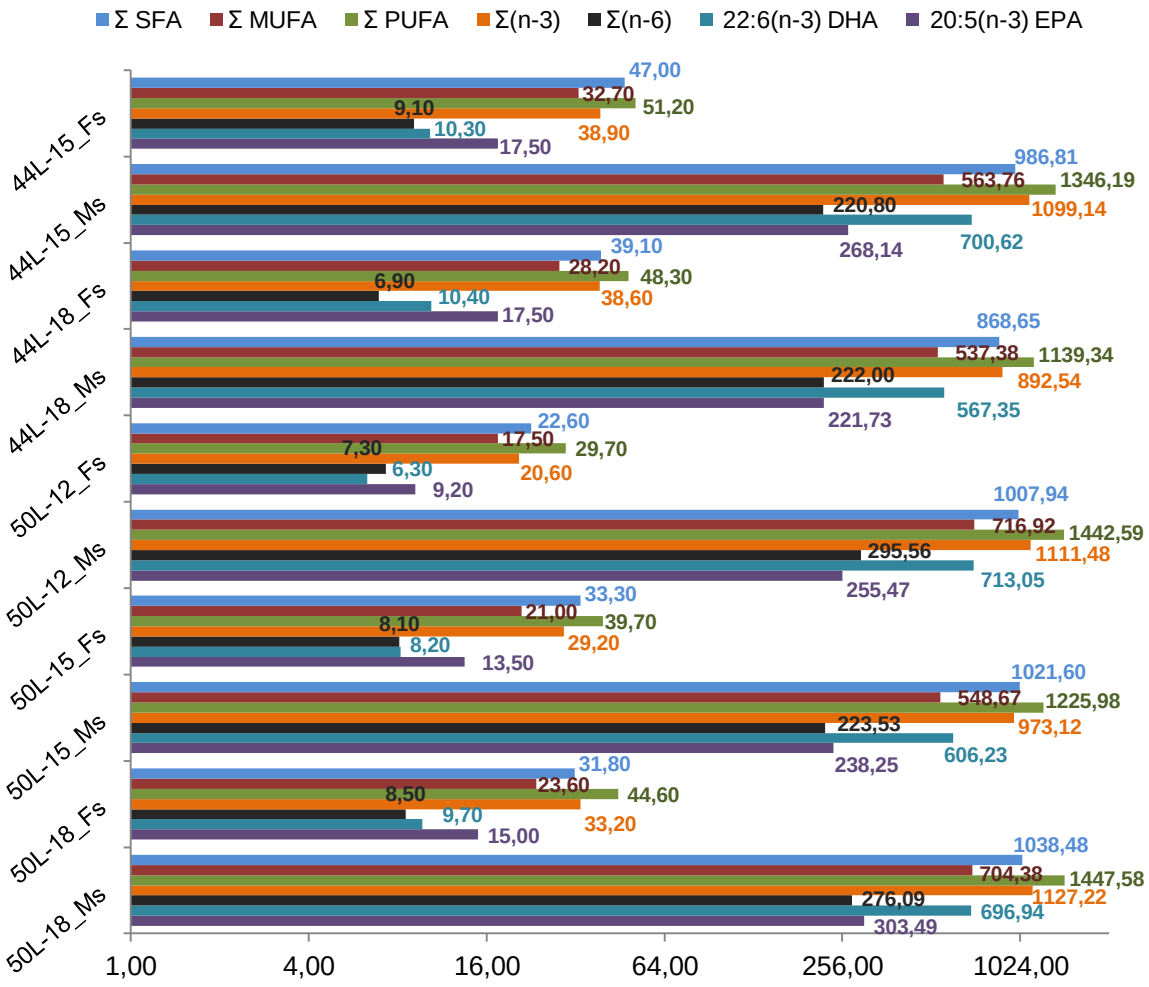


Figure 3.11 – Fatty acid profile in total SFA, MUFA, PUFA, (n-3), (n-6), EPA and DHA, of the feed samples (represented as %proteinL%lipid_Fs) and muscle samples (represented as %proteinL%lipid_Ms). Mean values (n=3) are represented in mg/100g of feed and muscle sample.

When the Atherogenicity and Thrombogenicity indexes (AI and TI, respectively) are calculated (**Table 3.6**), and applying the one-way ANOVA, no significant differences are found (p >0,05) between treatments. Nevertheless, treatment **50L-12** possesses the lowest IA (0,014) and the lowest IT (0,241).

Table 3.6 – Atherogenicity and Thrombogenicity indexes of the five different treatments.

	Dietary treatments				
	44L-15	44L-18	50L-12	50L-15	50L-18
Indexes					
Atherogenicity	0,459	0,480	0,422	0,528	0,491
Thrombogenicity	0,245	0,255	0,241	0,288	0,244

3.7 Feeding behavior

Meagre adapts very easily to captivity, nevertheless, some measures had to be taken. Since it gets "scared" with ease, each fiberglass tank was covered with protection nets (**Figure 3.12**), to prevent fishes from jumping outside the tanks. They are sensitive to shades and noises, and can get stressed with no difficult. Since meagre fish possesses a voracious appetite, feeding follows a pattern. When feeds are on the top of the water, and after hydration, they start to dive towards the bottom, meagre prefers to feed mostly in the middle, and less preference for the bottom of the tank.

Figure 3.12 – Fiberglass tank with experimental fishes, and the safety net on top, to prevent deaths.

4 DISCUSSION

There is a very limited knowledge on dietary nutrient requirements of meagre. The assessment of a optimum dietary protein:lipid ratio is essential to maximize production output and reduce costs. In a culture environment, the replication of wild conditions is made with ease, but feeds still represent the most important variable. With low dietary protein available, growth of meagre may be retarded, and with high content in dietary protein, feed will become very expensive and nitrogenous waste would be produced (**NRC, 1993**). The same stands for dietary lipids – deficiency makes the other nutrients (mainly protein) to be used for body maintenance and energy production, retarding growth, while excess of lipids may result in excessive fat deposition in visceral cavity and tissues. Many authors have found this excessive lipid deposition when studying the Atlantic halibut (*Hippoglossus hipoglossus*) (**Martins *et al*., 2007**), red drum (*Sciaenops ocellata*) (**Craig *et al*., 1999**), brown meagre (*Sciaena umbra*) (**Cakli *et al*., 2006**; **Chatzifotis *et al*., 2006**) and dusky kob (*Argyrosomus japonicus*) (**Bernatzeder *et al*., 2010**; **Woolley *et al*., 2010**).

Studies argue that sciaenids grow faster in cages than in tanks (**Ortega and Gándara, 2007; Piccolo *et al*., 2008; Cárdenas, 2010; Chatzifotis *et al*., 2011**), so growth parameters must be calculated and compared, to assess the efficacy of treatments. In

general, feed **50L18** presented the best performance when compared with the other treatments, since it is within the desired levels of dietary protein (DP) and dietary lipid (DL), referred by **Cárdenas (2010)**: DP>45% and DL≈17%. On the other way, feed **50L12** had the worst performance, mainly because of the low levels of dietary lipids. The reference levels for DP and DL were used in many studies (**Chatzifotis *et al*., 2006; Woolley *et al*., 2010; Chatzifotis *et al*., 2011; Martínez-Llorens *et al*., 2011**). The compliance with those values allows SGR rates to be higher than 1%/day, with feeds **50L15** and **50L18** showing the highest SGR's (0,99±0,04 and 1,21±0,00, respectively), which are comparable to the values obtained by **Woolley *et al*. (2010)**, **Chatzifotis *et al*. (2011)** and **Martínez-Llorens *et al*. (2011)**. Within the 50% DP group of treatments, **50L18** showed the highest PER (2,33±0,09), and the lowest FCR (1.04±0,04), showing that high values of DP and DL makes meagre to grow more (**Chatzifotis *et al*., 2011**), making the efficiency of feeds to be higher.

The increase of dietary lipid within the 50% DP group appears to have a spare protein effect (**Chatzifotis *et al*., 2011**), but when analyzing the 44% DP group, it is notable that the increase in dietary lipids (from **44L15** to **44L18**) actually diminishes PER means values, so the protein sparing effect by the increase of dietary lipids cannot be confirmed. High values of PER are related with high values of feed efficiency and high weight increase (**Thoman et al., 1999**), situation confirmed with treatments with 50% DP, when compared with 44% DP treatments.

The values of FCR are correlated to the amount of food ingested versus the fish weight. With similar amounts of ingested feed, treatment **50L18** allowed fishes to grow more, and therefore, to present lower FCR than the other treatments. **Chatzifotis *et al*. (2010)** and **Martínez-Llorens *et al*. (2011)** obtained similar results with feeds having the same values of group 44% DP, having poor growth together with high feeding rates, making mean values of FCR being higher than 1,38, which is according to the values obtained in this trial.

Finally, the high levels of dietary lipid inclusion make feeds more palatability acceptable (**Estévez et al., 2011**), and that fact could be confirmed by the high VFI recorded in diets **44L18**, **50L15** and **50L18**.

The retention values of dry matter (DM), protein and energy were higher in fishes fed treatment **50L18**, accompanied by high daily gain mean values of nitrogen (N) and energy. The high amount of protein present in the 50% DP group allowed fishes to retain more protein than the 44% DP group, showing that feeds for meagre should have around 50% of crude protein in their formulation (**Chatzifotis *et al*., 2011**), which also allows fishes to retain more N per kg of body weight.

All feeds were isoenergetic, and retention of energy was not different among treatments, with similar results being recorded by **Woolley *et al*. (2010)**. On a daily gain basis, diet **50L18** allowed fishes to gain more energy, opposite with diet **50L12**, which presented the lowest value (0,51±0,16 kJ) recorded. An absence of energy gain could be interpreted as a diet poor in nutrients (especially dietary lipids) allied with a very low feed intake.

The retention of dietary lipids was not statistically different among treatments, mainly due to the big variation among the data set, but some approximations may be inferred. The balance of dietary protein with dietary lipid in each diet influences the percentage of lipids that are retained, showing that the level of 12% DL is not appropriated for meagre

(**Chatzifotis *et al*., 2010; Chatzifotis *et al*., 2011**), so diet **50L12** should not be used in formulation of commercial feeds.

Similarly to what has been found in other cultured species, the proximate composition of meagre is affected by endogenous factors such as fish size and exogenous factors such as diet composition (**Chatzifotis *et al*., 2011**). On a whole body composition basis, and regarding dry matter values, feeds **44L15** and **44L18** presented high mean values for DM, mainly due to its lower content in DP, when compared with the 50% DP group.

The value of dry matter protein was lower with treatment **44L18**, since the interaction between an high level of DL and a low level of DP makes the levels of DP in fishes to be lower. The dry matter lipid was higher in the 44% DP group, since both DP and DL levels influence the amount of lipids present in fishes, with treatment **50L12** to have the lowest mean values, mainly due to the low lipid retention recorded. Its value is even lower than the initial one, what can be interpreted as the bad performance of this feed.

When on a fresh matter basis, moisture values of 50% DP group were higher than 44L18, mainly due to the low protein content in treatments 44% DP. Moisture and lipid values tend to have an inverse correlation (**Cakli *et al*., 2006**), and that was notable in the level of lipid in fishes fed diet **44L18** (6,53±0,94 %), opposing with fishes fed diet **50L12** (4,57±0,90 %), and when accounting moisture values, the ones of diet **50L12** (72,85±0,75 %) were higher than **44L18** (71,16±0,65 %), being these values similar to ones obtained by **Chatzifotis *et al*. (2010)**.

Regarding energy levels, diet **44L18** presented higher value when compared with **50L12**, mainly due to the different lipid content in the feeds, since high amounts of lipids could be used as an energy resource (**Chatzifotis *et al*., 2006**). Values of ash and protein were similar between all treatments, and in accordance with the study made by **Chatzifotis *et al*. (2010)**.

The lipid content of liver was higher in treatment **50L18** (63,11±3,98 %) than in treatment **50L12** (53,01±7,61 %), but similar to treatment **44L18** (58,26±10,05 %). The values of muscle lipid are not significant different between treatments, with an average value of 2,3%. These values are much higher than the ones obtained with brown meagre and red drum (**Chatzifotis *et al*., 2006**), regarding the lipid content of liver, but similar to the values obtained for muscle fat, confirming that meagre is a low fat fish. These findings prove the minor role of muscle as an energy depot, but liver still is the main energy central that fulfills meagre energy needs. Nevertheless, when dissecting fishes from treatment **50L18**, there were fat mesenteric depots within the abdominal wall, so fat was being accumulated in two places, and that it is not desirable.

The obtained digestive tract fat percentages, ranging from 17,56±1,73 (%) to 23,64±3,00 (%), were abnormal, and can only be due to errors in the dissection of the viscera. Since the viscera does not represent a fat storage depot, no conclusions could be made with the obtained data.

The HSI between treatments showed no differences, but the mean value was 2,45, being higher than the ones obtained in the studies made by **Piccolo *et al*. (2008)** and **Chatzifotis *et al*. (2010)**, mainly due to the high lipid content of livers, but no correlation was made between HSI and dietary nutrient levels (**Woolley *et al*., 2006**).

The fatty acid profile of the different treatments presented no significant differences, but the mean values of SFA (≈32%), MUFA (≈20%), PUFA (≈43%) and the ratio (n-3)/(n-6)

(≈4.2), are in accordance to the values referred by **Poli *et al*. (2003)**, **Cárdenas (2010)**, **Grigorakis *et al*. (2011)** and **Martínez-Llorens *et al*. (2011)**. The values for the (n-3)/(n-6) ratio in this trial are better than the ones obtained by **Lenas and Nathanailides (2011)**, when working with wild sea bass, with meagre presenting more omega-3. These findings, together with the low AI and TI, proves that meagre is an excellent source of omega-3 fatty acids, essential to a good heart condition and overall health benefits.

5 CONCLUSION

The main purpose of experimental trials within aquaculture is to create new techniques and feeding protocols, minimizing costs and maximizing production. The five experimental feeds had differences that may argue that some are not appropriate for the breading of meagre. Feeds with low dietary lipid, such as diet **50L12** should not be used for meagre aquaculture, since it doesn't supply fishes with the necessary protein and lipids for a optimum growth. Since the balance of dietary protein and dietary lipid is essential for an optimum growth, diet **50L18** was the best one for the meagre growout, but its value of dietary lipid should be rearranged, since some fishes would gain unwanted mesenteric fat. Diet **44L18** had a good performance, but its low level of dietary protein is a drawback for the rearing of meagre. Diet **50L15** had similar values to diet 50L18, not possessing significant differences, and without the problems with mesenteric fat deposition.

In a future study, diets should take in account the value of 50% dietary protein, and values between 15% and 18% of dietary lipid. Nevertheless, protein is still the most expensive ingredient when formulating feeds, but these values seem to be a good approach for an optimum diet that could maximize meagre growth.

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