MESTRADO CIÊNCIAS DO MAR - RECURSOS MARINHOS

ESPECIALIZAÇÃO AQUACULTURA E PESCAS

Impact of defatted mealworm larvae meal on European seabass *(Dicentrarchus labrax)* flesh quality.

Andreia da Silva Sousa

M

2020

Andreia da Silva Sousa. Impact of defatted mealworm larvae meal on European seabass (*Dicentrarchus labrax*) flesh quality.

M.ICBAS 2020

Impact of defatted mealworm larvae meal on European seabass (*Dicentrarchus labrax*) flesh quality.

Andreia da Silva Sousa

INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

Andreia da Silva Sousa

Impact of defatted mealworm larvae meal on European seabass (*Dicentrarchus labrax*) flesh quality

Dissertation for the Master Degree in Marine Sciences – Marine Biology submitted to the Abel Salazar Institute of Biomedical Sciences from the University of Porto.

Supervisor: Professor Doctor Luísa Maria Pinheiro Valente.
Category: Associate Professor.
Affiliation: Abel Salazar Institute of Biomedical Sciences of the University of Porto.
Interdisciplinary Centre of Marine and Environmental Research – CIIMAR.

Acknowledgements

First of all, I would like to express my most sincere gratitude to **Prof. Dra. Luísa Valente**, for all the opportunities she has been giving me over the years in my academic path as a trainee in bachelor's and master's degrees and also in my professional career, allowing me to be part of a project as a research fellow. Thank you for believing in me and for all the scientific knowledge of excellence that you have been transmitting to me. For all this, I will be forever grateful.

Secondly, I cannot help thank two special people: **Ana Basto**, for being my guidance in the last years, for helping me from my first day to the last, for all the patience to teach me everything, for the opportunity to let me be part of your project, and for all the friendship, thank you; **Alexandra Marques**, for having taught me everything in the laboratory, for all the fellowship and friendship, for always knowing how to answer my doubts, no matter how silly they might be. This work would not be possible without the two of you, both sharpened as a little researcher and I hope one day I will be able to repay all the help and affection you have given me. Thank you both!

To **LANUCE members**, I want to thank everyone with all my heart, for all the wisdom in the most diverse areas that you have given me, for the help and all the moments, good or bad, that we have been going through together, certainly made these years unforgettable. Special thanks to Tiago and David for all the help in the last moments of this journey. To Inês, Mariana, Luís, Cátia, Olivia, Cristina, Ricardo, Daniela, Vera e Beatriz thank you very much.

To **Prof. Dr. Eduardo Rocha** and all the master colleagues, specially Dionísio, Medina, Ivan, and Juliana. Thank you for all the support in the last two years, it was a pleasure to meet you and finish this journey with you.

To my dear “migas”, Lara, Filipa, Bruna, Maria, and Marisa. You are everything that I always envisioned in a friendship, you were always with me from the first day in college and for sure this will be just a chapter that we travel together. Thank you for all we lived together, to share with me the absolute best times of my life, for the encouragement in the most difficult times, and for being who you are. For this and for all that is yet to come I am eternally grateful.

To “slowly lowly”, Nuno, Dani, Inês, Ju, and César. Thank you for all the fun moments we have been passed together, but mainly for helping me to grow as a person, to overcome my fears, and to be much of what I am today. I am so happy to have shared these years with you

and to have seen all of our personal development. I look forward to seeing all that we will one day become and to spend the next stages of life together. Thank you for everything.

A big thanks to my family, especially to my parents that were always concern about “my fish”. Thank you so much for always believing in me and my dreams, letting me be who I am, and for constantly supporting me no matter what during the last years, even when the choices were not easy. To my brother, who probably does not quite understand what I do in my work, but who nonetheless supports me unconditionally. Thank you for being the best brother and for always making me laugh with our conversations. For all the challenges that we overcame together and for all that you gave me, thank you both for being my role models. Your unconditional love and support have made me who I am, and it reflects on these pages.

Last but not least, a special thanks to João, for being my safety spot wherever you are. Thank you for constantly making me see what I am capable of, for carefully listening to my worries, concerns, and especially for being as much crazy as I am. Thank you for all your support; you are my foundation throughout all my challenges.

Abstract

The expansion of the aquaculture sector and overexploitation of marine resources led to a global reduction of the use of fish meal (FM) and fish oil in industrially compounded aquafeeds. The insect meal (IM) is a rich source of protein with a well-balanced amino acid profile, lipids, and vitamins, and was approved by the European Union in 2017, being considered an alternative protein source for aquafeeds.

This work aimed to evaluate the feasibility of replacing increasing levels of FM (0, 50, and 100%) by defatted *Tenebrio molitor* (TM) larvae meal in European seabass (*Dicentrarchus labrax*) diets. Each dietary treatment was assigned to quadruplicate homogeneous groups of 15 fish that were fed for 16 weeks. By the end of the experiment, several characteristics of the fillet quality were evaluated in 12 fish per treatment. The nutritional value of fish carcass and muscle was evaluated after chemical determination of total lipids and fatty acids profile. The fillet was also evaluated in terms of colour, texture, and global acceptance by a sensory panel.

Overall, results were promising for all diets tested, but when compared to control (CTRL), the diet where 50% FM was replaced by TM (TM50) resulted in the best feed conversion ratio and protein efficiency rate, indicating a better use of this diet by the fish. In terms of fatty acid retention, there were no major differences amongst diets. Muscle had a greater deposition of saturated fatty acids with increased inclusion of insects in the diet, but, on the contrary, there were no significant differences in the sum of monounsaturated and polyunsaturated fatty acids. There was also a decrease in the n-3 / n-6 ratio with TM inclusion. Despite the reduction of the relative content of EPA and DHA (% total fatty acids) in the muscle, the absolute values of EPA + DHA in a fillet portion of 100 g for human consumption remained above the recommended levels for human consumption (>0.25g / 100g of wet weight) in all fish and did not vary significantly among treatments. These results suggest a possible partial replacement of fish meal with *Tenebrio molitor* in diets for European seabass.

Keywords: aquaculture; seabass; insect flour; *Tenebrio molitor*; alternative protein sources; fatty acids

Resumo

A expansão do setor da aquacultura e a exploração de recursos marinhos levaram a uma diminuição global da utilização de farinha e óleo de peixe na produção industrial de rações. A farinha de inseto é um ingrediente rico em proteína, com um perfil de aminoácidos equilibrado, lipídios e vitaminas. Foi aprovada pela União Europeia em 2017, sendo considerada uma fonte proteica alternativa em rações para peixes.

Este trabalho visou avaliar a viabilidade de substituir a FM por níveis crescentes (0, 50 e 100%) de farinha de inseto *Tenebrio molitor* desengordurada em dietas para robalo europeu (*Dicentrarchus labrax*). Cada tratamento dietético foi distribuído em quadruplicado por grupos homogéneos de 15 peixes que foram alimentados durante 16 semanas. No final do ensaio, várias características da qualidade do filete foram avaliadas em 12 peixes por tratamento. O valor nutricional da carcaça e músculo dos peixes foi avaliado após determinação química dos lipídios totais e do perfil ácidos gordos. Foi ainda realizada uma avaliação sensorial do filete através da cor, textura e aceitação global recorrendo a um painel sensorial de consumidores.

No geral, os resultados foram promissores para todas as dietas testadas, mas quando comparadas com o CTRL a dieta que obteve melhores resultados foi a dieta onde se substituiu 50% da FM por TM (TM50) que resultou numa melhor taxa de conversão alimentar e eficiência proteica, indicando uma melhor utilização da dieta TM50 por parte dos peixes. O músculo teve uma maior deposição de ácidos gordos saturados com o aumento de inclusão de insetos na dieta, mas, pelo contrário, não houve diferenças significativas no somatório de ácidos gordos monoinsaturados e polinsaturados. Também houve uma diminuição no rácio n-3 / n-6 com a inclusão de TM. Apesar da diminuição da % relativa de EPA e DHA (% ácidos gordos) no músculo, os valores absolutos de EPA + DHA num filete de 100 g mantiveram-se acima dos níveis recomendados para consumo humano (>0.25 g/ 100 g de peso fresco) em todos os peixes e não variando significativamente entre tratamentos. Estes resultados indicam a possibilidade de uma futura substituição parcial de farinha de peixe por farinha de inseto *Tenebrio molitor* em dietas para robalo.

Palavras chave: aquacultura; robalo; farinha de insetos; *Tenebrio molitor*; fontes proteicas alternativas; ácidos gordos

Content Index

List of Abbreviations

ABW – Average body weight

ALA – Alpha-linolenic acid

BSF – Black soldier fly

CF – Crude fat

CP – Crude protein

CTRL – Control diet

DHA – Docosahexaenoic acid

DPA – Docosapentaenoic acid

DM – Dry matter

EPA – Eicosapentaenoic acid

FA – Fatty acids

FCR – Fish conversion ratio

FM – Fish meal

HSI – Hepatosomatic index

K factor – Condition factor

LC-PUFA – Long-chain polyunsaturated fatty acids

MD – *Musca domestica*

MUFA – Monounsaturated fatty acids

PER – Protein efficiency ratio

PUFA - Polyunsaturated fatty acids

SGR – Specific growth rate

SFA – Saturated fatty acids

TM – *Tenebrio molitor*

TM50 – 50% of fish meal replacement by defatted *Tenebrio molitor* larvae

TM100 – 100% of fish meal replacement by defatted *Tenebrio molitor* larvae

TMd – Deffated *Tenebrio molitor* larvae

VFI – Voluntary feed intake

VSI – Viscerosomatic index

WW – Wet weight

Figure Index

Tables Index

Introductory note

This work is supported by the Project ANIMAL4AQUA, funded by Portugal 2020, financed by the European Regional Development Fund (FEDER) through the Operational Competitiveness Program (COMPETE) - POCI-01-0247-FEDER – 017610.

To promote work dissemination, a poster was presented at 13th edition Meeting Youth Research at the University of Porto (IJUP) that took place on February 12-14 at the University of Porto Rectory.

UNIÃO EUROPEIA
Fundo Europeu de
Desenvolvimento Regional

COMPETE 2020
PROGRAMA OPERACIONAL COMPETITIVIDADE E INTERNACIONALIZAÇÃO

PORTUGAL 2020

Declaração de Honra

Declaro que a presente dissertação é da minha autoria e não foi utilizada previamente noutro curso ou unidade curricular desta, ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras de atribuição, e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referenciação. Tenho consciência que a prática de plágio e auto plágio constitui um ilícito académico.

ANDREIA DA SILVA SOUSA

1. Introduction

1. 1. Aquaculture role in fish supply: numbers and trends

Currently, aquaculture is the fastest-growing food production sector and plays an important role in fish supply for human consumption. In 2018, approximately 88% of the total fish production was directly used for human consumption and only 12% was used for non-food purposes. In 2018, global aquaculture production reached 114.5 million tonnes (including aquatic algae, ornamental seashells and pearls production), with finfish production being responsible for 54.3 million tonnes, followed by molluscs and crustaceans production with 17.7 and 9.4 million tonnes, making the aquaculture sector the fastest growing one in food production (Figure 1) (Clavelle et al., 2019; FAO, 2020).

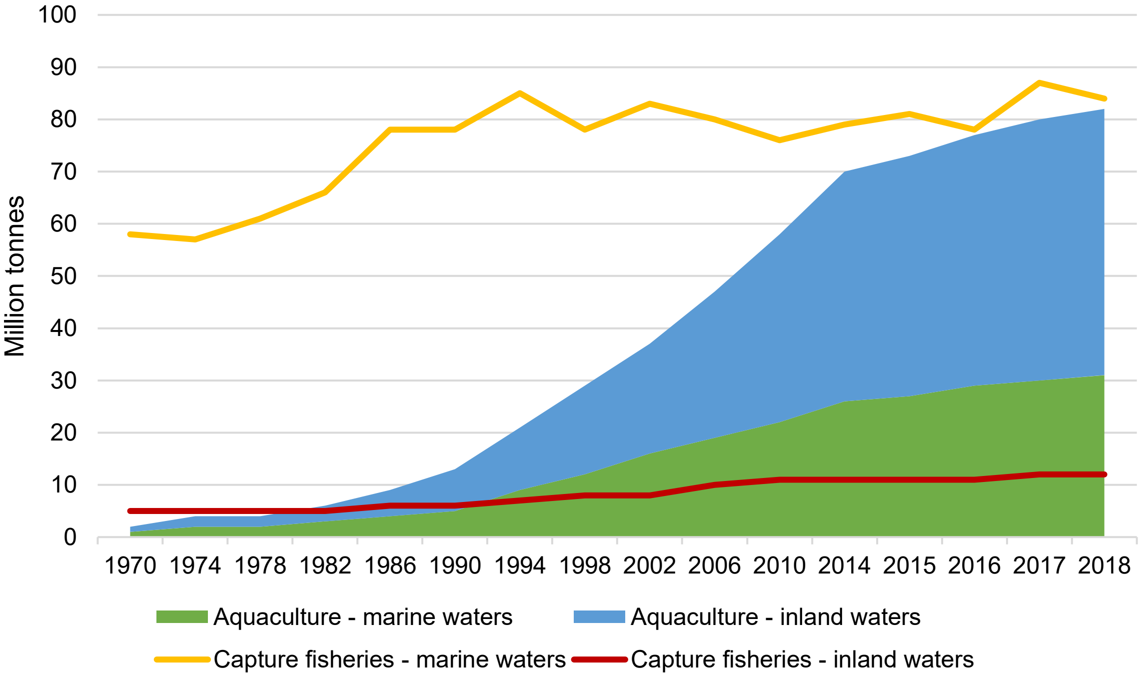

Figure 1. World capture fisheries and aquaculture production. Retrieved from: FAO (2020).

Asia leads the world aquaculture production accounting for 74% of total world production. However, this percentage has been decreasing due to the rise of aquaculture production in

Europe. In 2017, aquaculture production in the European Union reached 1.37 million tonnes, the highest value of the last 10 years (EUMOFA, 2019a). This increase in volume represented a value of EUR 5.06 billion and is related to several factors such as the strengthening in the economic value of some of the most commercialized species due to their high demand (EUMOFA, 2019a). Another significant remark is related to aquaculture entrepreneurship. According to FAO (2018), 19.3 million people were employed in the aquaculture sector, contrary to the fisheries sector that decreased their employees by 68% between 1990 and 2016.

Regarding aquaculture species, grass carp (*Ctenopharyngon idellus*), silver carp (*Hypophthalmichthys molitrix*), and Nile tilapia (*Oreochromis niloticus*) were the top three species produced in the world in 2018 (5704.0, 4788.5 and 4525.4 thousand tonnes, respectively) (FAO, 2020). Regarding the European Union, the values of aquaculture production by main commercial species are represented in Figure 2. The top five producers in the EU were Spain, the United Kingdom (UK, values before Brexit), France, Italy, and Greece. Other countries as Portugal and Malta had a remarkable increase in their production of oyster and bluefin tuna, respectively.

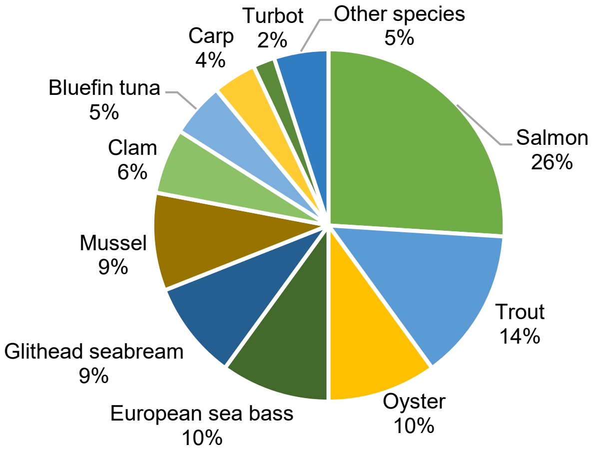

Figure 2. Composition of EU aquaculture production by main commercial species (in value): Retrieved from: EUMOFA (2019a).

The species with higher commercial value in EU was by far Atlantic salmon (*Salmo salar)*, followed by trout. Most of the salmon production occurs in the UK (90%), with market values around 6.32 EUR/kg. Trout production takes place mainly in Italy (18%), France (19%), and Denmark (16%), with an average price of 3.53 EUR/kg. Pacific cupped oyster (*Crassostrea gigas*) is the main oyster species produced in the EU, dominated by France and with a market price of 4.97 EUR/kg (EUMOFA, 2019a). Regarding other main commercial species, European seabass is a marine fish species with high commercial value in Southern Europe and an important economic impact in Mediterranean countries. In 2017, the EU European seabass production account for 79.102 tonnes, around 490 million € (EUMOFA, 2019a). Spain is the top EU aquaculture producer, and this is mainly related to seabass production. They are followed by Greece, due to their dominance in exports among EU trade (62% of all intra-EU exports in 2016), counting with 44285 tonnes of production valued as 5.59 EUR/kg, which worth 248 million € in 2017 (EUMOFA, 2019b). It is important to highlight that seabass production in Spain increased by 125% in the last 10 years, a remarkable increment, and emphasizing its importance in aquaculture production (EUMOFA, 2019a).

1. 2. The relationship between consumers and the aquaculture fish market

Nowadays, fish consumption represents 17% of the global population's intake of animal proteins and fish consumption per capita increased from 9.0 kg in 1961 to 20.5 kg in 2018 (FAO, 2020). In 2017, the EU consumption of fish and seafood was 12.45 million tonnes in live weight, meaning a *per capita* consumption of 24.35 kg. Although aquaculture was only responsible for 6.35 kg, this value has been increasing. Fish stocks are overexploited and aquaculture as the fastest growing animal production sector plays, now more than ever, and an important role in fish supply for human consumption (EUMOFA, 2019a). Portugal is the main fish consumer *per capita* in the EU (56.8 kg/year), and the member state with the most balanced ratio between fish and meat consumption, followed by Spain, Malta and Luxembourg (Figure 3). On the contrary, Hungary is the consumer with the lowest consumption *per capita* in the EU, followed by Bulgaria, Romania, and the Czech Republic (EUMOFA, 2019a; Hua et al. 2019).

In 2017, a study was conducted to evaluate the consumers' purchase preference between farmed or wild fish. It was concluded that wild fish is significantly desired at the EU level, and 34% of the population prefers wild fish over farmed products (8%). This predilection shows different values among age classes, where younger people tend to prefer farmed fish while older people prefer wild fish (European Commission, 2017a). Moreover, another study developed by Claret et al. (2016), has demonstrated at least on this date that under blind conditions consumers prefer farmed fish but, when informed, their preference goes to wild fish. This happens because there are several factors influencing food choices, in particular fish choices. Consumers' choices may change not only due to their preferences, economic, and life status but also due to product characteristics, such as sensory and nutritional quality and price (Thong and Solgaard, 2017). The frequency of consumption of aquaculture products is positively correlated with the age of consumers, where, the older the person, the higher frequency of consumption (Morales and Higuchi, 2018). It is also positively related to socioeconomic status, where people with higher socioeconomic status consume fish aquaculture products more often than lower-class citizens such as students and workers (European Commission, 2017b; Thong and Solgaard, 2017).

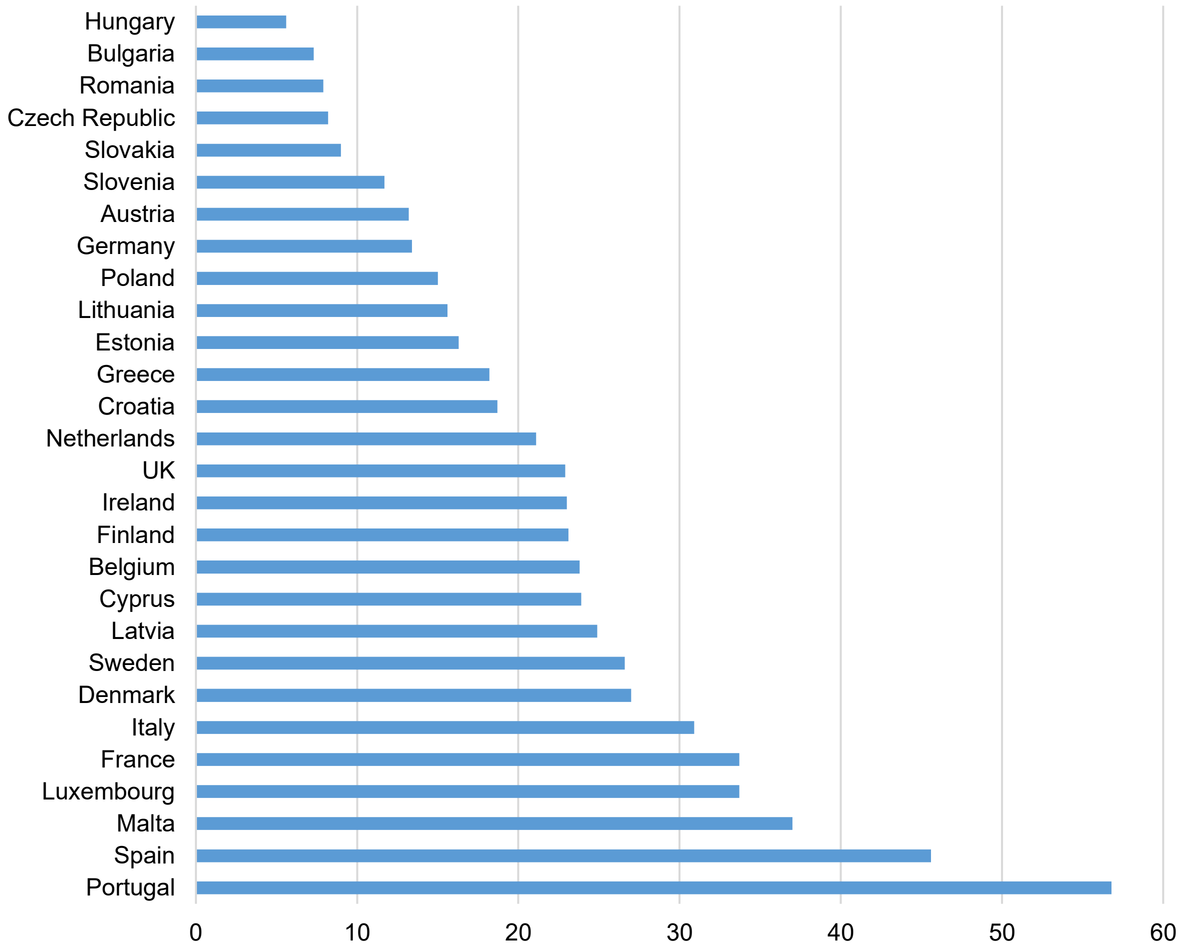

Figure 3. Per capita apparent consumption of fisheries and aquaculture products by EU members (kg live weight/capita/year). Retrieved from: EUMOFA (2019a).

Among the non-consumers of aquaculture fish products, the major issue is related to the organoleptic specifics of seafood such as the taste, smell and appearance, as well as the emergence of new ideologies that affect feeding like veganism or vegetarianism (European Commission, 2017). A study conducted by Ruiz-Chico et al. (2020) regarding the social acceptance of aquaculture products by Spanish consumers revealed that some of the respondents (19.69%) are concerned with the abusive feeds and/or chemicals in operating companies, and also pointed out the quality and taste of the fish (8.46%). However, if safety is ensured, the consumer is willing to pay more for aquaculture products than those from the sea, making food safety an important factor in aquaculture product choice (Ruiz-Chico et al. 2020). Another study carried out by Pieniak et al. (2013) revealed that 68.5% of the participants (European participants) affirm that the phrase ‘‘Farmed fish contains more mercury than wild fish’’ is true, indicating that stigmas may be one of the major problems in aquaculture impact in fish purchasers.

1. 3. The importance of fish for human consumption

Fish has optimal levels of digestible protein, peptides, essential amino acids, vitamins (A; B12; D and E) minerals (iodine and selenium), bioactive compounds (taurine; phytosterols; antioxidants and phospholipids), and essential fatty acids (EFA) (Elvevoll et al., 2006; Kwasek et al., 2020; Larsen et al., 2011; Nogales-Mérida et al., 2019; Tocher, 2015).

Fatty acids include saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The saturated fatty acids are directly related to cardiovascular diseases (Larsen et al., 2011; Swanson et al., 2012). Regarding the omega-3 long-chain PUFA (LC-PUFA), they are the most beneficial to health, due to their anti-inflammatory properties, improvements in cardiovascular functions and Alzheimer's prevention. The advantage of fish consumption by humans is mainly due to the presence of omega-3 LC-PUFA, eicosapentaenoic acid (C20:5n-3; EPA) and docosahexaenoic acid (C22:6n-3; DHA) (Figure 3) (Kwasek et al., 2020). These two EFAs can be obtained by direct fish consumption or synthesized through alpha-linolenic acid (C18:3n-3; ALA), but it is important to note that this endogenous synthesis of omega 3 and 6 does not occur in most species, including humans, so the only way to obtain them is through the direct dietary intake (Alvergne et al., 2016; FAO, 2010; Lund, 2013; Mozaffarian and Wu, 2012). Recommended daily intake of EPA and DHA by EFSA generally ranges from 250 mg to 500 mg per day for adults, with an additional 200 mg of DHA per day for pregnant and breastfeeding women. Patients with heart disease are recommended to take 1 g of EPA and DHA daily, while those with hyperglycaemia are advised to take 2 to 4 g of EPA and DHA daily (EFSA, 2012; Lichtenstein et al., 2006; Sardesai, 2020).

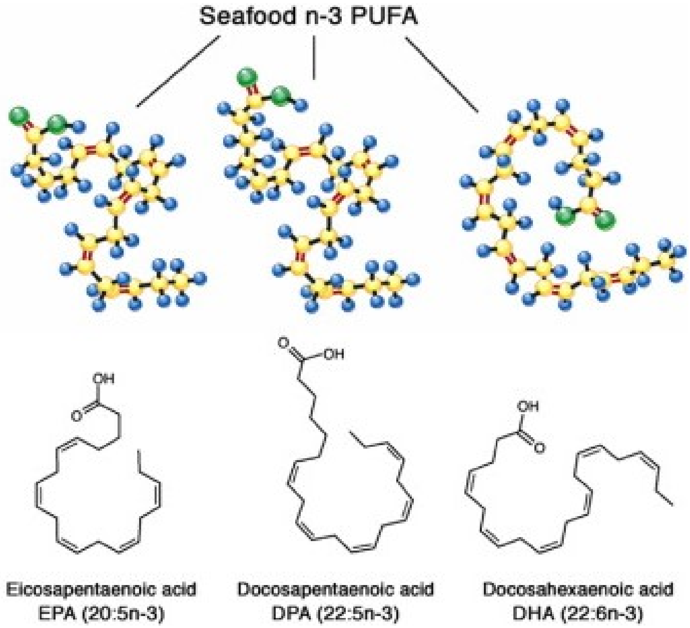

Figure 4. Structure of n-3 PUFA. Adapted from: Mozaffarian and Wu (2012).

The major benefits of n-3 PUFA consumption are associated with the cardiovascular system since they play a major role in preventing heart diseases (Elvevoll et al., 2006; Kris-Etherton et al., 2002; Larsen et al., 2011). This happens because the substitution of SFA for PUFA decreases the low-density lipoprotein (LDL) cholesterol concentration, known as the "bad cholesterol" and consequently also decreases the risk of heart disease (Burlingame et al. 2009; FAO, 2010). Moreover, Alvergne et al. (2016) reported that in addition to heart disease, fish-based diets with high concentrations of n-3 LC-PUFA may be associated with a decreased onset of cancer, inflammatory and immune diseases. Concerning psychological disorders, EPA and DHA may prevent the development of depression, attention deficit, hyperactivity disorder and dementia (FAO, 2010; Janssen and Kiliaan 2014; Larsen et al., 2011; Tacon and Metian, 2013). Brain, retina, and neural tissues are rich in LC-PUFA and so, there are some special recommendations for fatty acids intake in the early stages of life as a fetus, infant, and children, to ensure the proper development of the eyes and brain (Janssen and Kiliaan, 2014; Larsen et al., 2011; Lund 2013; Tacon and Metian, 2013).

The consumption of fish is important to meet EPA and DHA daily requirements. The EPA and DHA quantities found in the different fish/shellfish species are presented in Table 1. Normally, the health benefits of fish consumption are related to lipids effects, but proteins and peptides also have nutritional significance depending on their amino acid composition, length and structure. Boukortt et al. (2004) demonstrated that fish proteins have a positive impact on controlling diabetes since they enhance the antioxidant defences in kidney and heart, the first organs attacked by diabetes complications. Furthermore, fish protein stimulates the control of weight gain due to increased satiety and thermogenesis (Ait-Yahia et al., 2003; Larsen et al., 2011).

Table 1. Selected food sources of EPA and DHA content (g). Retrieved from: Sardesai (2020).

Source	EPA (g)*	DHA (g)*
Herring	1.06	0.75
Salmon	0.86	0.62
Sardines	0.45	0.74
Crab	0.24	0.10
Oysters	0.75	0.93
Tuna	0.40	0.44
Trout	0.45	0.74
Mackerel	0.43	0.59
Shrimp	0.07	0.10

*Values presented regarding serving sizes with 87.5 g.

1. 4. Importance of fillet quality

The nutritional value of fish muscle may vary according to numerous aspects, such as fish species, age, sexual maturity and size. Genetics and environmental conditions (oxygen concentration, temperature, photoperiod and pH) also play an important role in muscle development. The dietary composition is the main determinant of the nutritional content of fish muscle. The study of fish nutrition is important to improve knowledge and overcome the challenges posed by the increased product demand (Videler, 2011).

Fish muscle growth and development is a balance between muscle fibre hyperplasia and hypertrophy and elongation. This growth is divided into three phases: 1) the embryonic phase, 2) the late embryonic and early larval phase, and 3) the late larval and juvenile phase (Valente et al., 2013). In fish muscle, there are three different categories able to affect muscle' quality, the muscle fibres (made up of protein-rich myofibrils), cell membranes (formed by lipids as phospholipids), and connective tissue assembled by collagen (Kiessling et al., 2006). Fish axial muscle is organized into myotomes in red, pink, and white muscle (Figure 5). Red muscle is the superficial layer under the skin and uses aerobic metabolism. White muscle is the deep layer forming lateral muscles of fish using anaerobic metabolic pathways and represents 80-

95% of the edible portion of the muscle, depending on the species (Johnston, 2008; López-Albors et al., 2008). The intermediate layer, i.e. pink muscle, develops towards the end of larval life in some species (Johnston, 1999; Periago et al., 2005; Veggetti et al., 1990; Videler, 2011). The muscle colour is related to its composition; the red muscle has greater vascularization and its colour is due to the presence of myoglobin, which is not observed in the white muscle (Videler, 2011). Adipocytes are in the myoseptum, a structure of the connective tissue that can separate muscle layers and increase muscle growth. They are responsible for the storage of lipid content of the muscle that will further affect muscle colour and flavour (Johnston, 2008; Weil et al., 2013).

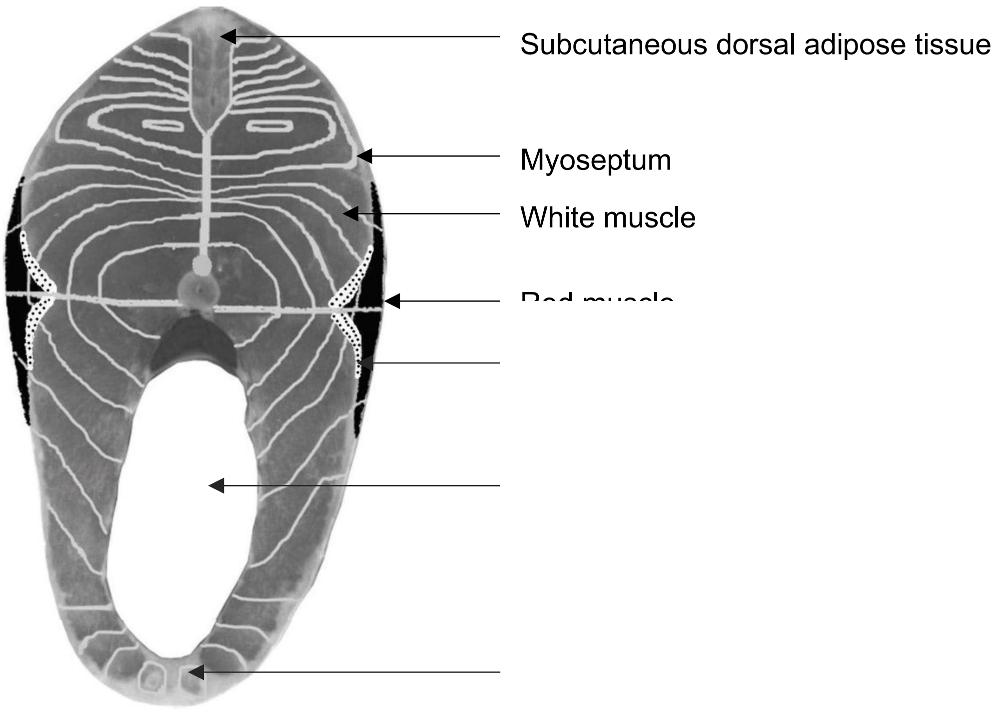

Figure 5. Diagrammatic organization and distribution of muscle. Adapted from: Listrat et al. (2016).

The study of fish sensory and nutritional characteristics is important for the fish industry since these properties will determine its acceptance by the consumer, especially in the white muscle (Coppes et al., 2002; Martinsdóttir, 2010). The most important properties of fish muscle to achieve consumers' satisfaction are the colour, texture, fat content, flavour (aromas), and chemical composition (Spence et al., 2010; Suman and Joseph, 2013). Colour and texture are influenced by intrinsic factors (species, size, and sexual maturity) and extrinsic factors (source of nutrients, season, water salinity, temperature, etc.) (Fuentes et al., 2010). The nutritional value and organoleptic characteristics of fish are especially affected by farming conditions;

artificial diets provide a wide range of nutrients, which determine not only the growth rate of the fish but also the composition of the flesh, in particular the lipid content, which can be modified both quantitatively and qualitatively (Izquierdo et al., 2003).

Flesh colour is important from a commercial point of view. The visual indicative cannot change the food taste directly, but it can influence the gustatory, olfactory, oral-somatosensory qualities, and the general perception of the flavour. Colour is the first stimulus for consumers through skin pigmentation or fish body shape (Spence et al., 2010; Suman and Joseph, 2013). Colour is an attribute whose evaluation becomes relevant in the quality control of products since it is an important sensory attribute of food that directly determines its acceptability (Gatlin et al., 2007). From the physical-chemical point of view, colour is the result of the interaction between a source of light and pigments, through which energy is absorbed and emitted as complementary non-absorbed radiation, in the region of wavelengths that human vision is capable of perceiving (Cairone et al., 2020; Clydesdale, 1991). Colour can be modified by the inclusion of pigments in dietary ingredients. The effects resulting from the inclusion of pigments in a diet on the colour of fillet have been widely described in fish with pigmented muscle, such as salmon (Belghit et al., 2018; Gatlin et al., 2007). For seabass, as it is a white fillet fish, it must be taken into account that the ingredients used in the formulation of the diets do not change the final colour of the product, as these changes may negatively affect the perception of the consumer (Li et al., 2007).

Texture and flavour are two of the most important measurements to assess flesh sensory evaluation since they can lead to a better consumer eating experience. Texture evaluation implicates mouth-feel, firmness, chewiness, juiciness and dryness that can be modified by enzymatic and chemical reactions, changes in elasticity or development of toughness (Bugeon et al., 2010; Coppes et al., 2002; Johnston, 2008). Firmness and fillet separation are determinants in consumer acceptability, one is related to muscle cutting problems and the other with post-mortem fracture of the connective tissue matrix amidst muscle fibres, respectively (Johnston, 2008). Muscle cellularity (number of fibres and diameter) simultaneously with the firmness of the raw flesh is an important determinant of the textural characteristics of the flesh, mainly of white muscle. Species with firmer texture had smaller fibres than species with softer texture (Ayala et al., 2005; Johnston, 1999; Periago et al., 2005). Collagen also affects fish texture due to its role in mechanical strength of the connective tissue matrix preserving all myotomes through collagen proteins (Cheng et al., 2014; Chéret et al., 2005; Johnston, 2008; Johnston et al., 2006; Torgersen et al., 2014). Lipids in fish flesh are divided into neutral and polar. Neutral lipids are triglycerides that are known for having a large quantity of LC-PUFA, and polar lipids are a steady content of fatty acids. They have a pivotal role in muscle texture since flesh fat content exceeding 18% (normally in diets with high lipidic energy) will decrease the texture (in this case, hardness) of fish fillet and processing characteristics (Johnston et al., 2006; Taylor et al., 2002; Weil et al., 2013).

1. 5. The importance of new protein sources in aquaculture feeds

The increase of aquaculture production is highly dependent on the increased production of aquafeeds, which are strongly dependent on FM as a major source of protein, particularly to produce carnivorous fish species (FAO, 2018). However, the world availability of FM is becoming scarce and its utilization competes not only with other animal feeds but also directly with human nutrition (Rana et al., 2009). According to EUMOFA (2019) data, each year 20 million tonnes of raw material are used to produce FM, leading to a evaluation and consequent increase in FM price. In the last twenty years, the FM price increased more than 400% and fish farming has grown more than 200% due to increased human consumption (Belforti et al., 2015; Nogales-Mérida et al., 2019). Thus, the sustainable development of aquaculture depends on the selection of alternative protein sources to FM (Campos et al., 2017).

FM is a rich source of protein due to its well-balanced amino acid profile and high palatability. FM is produced from wild fish, fish by-products and macroinvertebrates and consists of 60–72% protein and 5–12% fat, mainly LC-PUFA such as EPA and DHA (Shepherd and Jackson, 2013; Tacon et al., 2011). Also, FM is a great source of EFA, minerals (calcium, phosphorus, iron, zinc, selenium, and iodine), and vitamins (riboflavin, niacin, vitamins A and D), (Al-Qazzaz, and Ismail, 2016; Barroso et al., 2014).

Carnivorous fish species are the most produced fish in European aquaculture and FM total substitution is still an obstacle for the feed industry (Gasco et al., 2018; Ido et al., 2019). This fish species need large quantities of wild fish and, in this case, pelagic fish account for 70% of aquaculture production, leading to an unsustainable production due to the intensive use of natural fish stock resources (Froehlich et al., 2018; Hua et al., 2019). This issue has strong ecological impacts since forage fish catches reached their limits and affects directly higher trophic species as large fish, marine mammals, and seabirds since they're the bonding between them and the primary producers (Cashion et al., 2017; Clavelle et al., 2019).

1. 6. Insect meal: a possible substitute for fish meal

Recently, insects have been pointed out as a good protein source for human consumption, livestock, and fish feed (Bosch et al., 2019; Tinder et al., 2017; Huis and Oonincx, 2017). In 2017, the EU authorized the use of insect proteins from seven insect species (black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus assimilis*)) in aquafeeds (European Commission, 2017a).

There are advantages in using insects as feed ingredient (Tang et al., 2019). Arthropods like the shrimps have a large variety of species, having great biodiversity, short maturation periods with a high reproduction rate, which allows a regular production on a large scale. (Al-Qazzaz, and Ismail, 2016; Rumpold, and Schlüter, 2013). Moreover, the complete life cycle of an insect is much shorter when compared to other animals. This will benefit not only the production rate but also the possibility to genetically improve insects and select the more convenient lines (Huis, 2020).

Insects are poikilothermic animals, so that they don’t need energy to regulate body temperature and, consequently, they have more energy available to convert feed into body-weight, improving values of FCR (Halloran et al., 2016). It is estimated that broiler chickens, pork and beef requires approximately 2.5, 5.0 and 10.0 kg of feed, respectively, to gain 1 kg of weight, while insects only need 1.7 kg of feed to increase 1 kg of body mass (Huis, 2013). Another benefit is the fact that around 80% of an insect is edible and digestible (compared with 55% for broiler chickens and pigs and only 40% for beef), avoiding losses or possible costs in removing feathers, bones, cartilage, or fur/hair that normally don’t have high nutritional value (Koutsos et al., 2019; Parodi et al., 2018; Huis, 2013). Also, insects are part of some carnivorous fish species diet, which could mitigate the lack of FM for partial and total substitutions for these species (Gasco et al., 2018).

Insects are eco-friendly since their production has a low ecological footprint due to the fewer greenhouse gases emission, requirement of small land areas and need of little water consumption (Magalhães et al., 2017; Sogari et al., 2019). Approximately 80% of the global agriculture land and 29% of the water are used by livestock production (Weindl et al., 2017). For instance, to produce 1 kg of chicken, pork, or beef, a total of 1498, 2819, and 9678 litters of water are needed, respectively, unlike insects that need only 25 litters to produce the same amount (in these case, of mealworm) (Koutsos et al., 2019). The only greenhouse gases emission in insect production results from drying the larvae and feed manufacture, and even these can be minimized. For instance, mealworms release 20 times less methane and 50 times less nitrous oxide emissions than pigs (described per kg of body weight gain) (Parodi et al., 2018). It must be considered that insects have several food sources to grow on such as industrial waste (Varelas, 2019). Mealworm, for example, can be produced in organic waste such as fruits and vegetables, housefly can grow in dung but the species that can better perform in this type of growth is the black soldier fly that uses nearly every type of waste for growth (FAO, 2013; Rumpold and Schlüter 2013). This is a good opportunity to give livestock an additional value with biowaste degradation and using by-products converting them into food, feed, and fertilizers. They can also have a role in biodiversity conservation since they boost plant pollination and pest control (Giroud et al., 2016; Varelas, 2019).

1. 6. 1. Insects production and prices

Insect production has been increasing since it has been seen as a possible replacement of conventional animal ingredients in the various production areas and the numbers show that the insect feeds market increased by 14% between 2011 and 2015, with the large quantities directed to poultry, followed by pigs and fish production (Huis and Oonincx, 2017). Insect prices may vary greatly depending on the species concerned or the type of production associated. Their production is divided into three main categories, harvesting, semi-domestication (outdoor farming), and farming (indoor farming) (Varelas, 2019). Insects harvest from nature had no fixed price since there may be environmental fluctuations where they are caught (FAO, 2013). In these cases, several factors can increase prices such as intensive pesticide use, deforestation, and overall pollution. However, this method is not feasible due to the possibility of generating overexploitation of the species, leading to extinction and forest destruction, and due to the difficulty of controlling hygiene and sanitary conditions. In addition, these fluctuations could lead to a variation in the composition of flour that was made with these wild insects (FAO, 2013; Rumpold and Schlüter, 2013; Huis and Oonincx, 2017).

In Europe, more precisely in the Netherlands, 50 g of freeze-dried mealworms are sold for 4.58 € and so, the final product can cost up to 32 EUR/kg rehydrated weight. Many factors can change the price of insects, for instance, the life stage (larvae, pupae, adult), the amount and size of the order, the processing technologies (dried, frozen), and the stakeholders' type (retailers or wholesalers) so that, the price of black soldier fly may vary from 2 EUR/kg to 9

EUR/kg and mealworms from 15 EUR/kg to 32 EUR/kg (Rumpold and Schlüter, 2013). Nevertheless, for the EU economy, there are alternatives to decrease production costs, for example, using food waste and nonprofitable by-products from manufacturing procedures, achieving price advantage compared to importing FM and soybean (Grau et al., 2017).

Although insect meal price is generally higher than FM, efforts have been made to counter the trend. According to the Brabant Development Company, insect meal prices will compete with FM prices by the year 2023 (Arru et al., 2019). This will happen when insect production reaches around 80% of mechanization, leading to a decline of production costs as manual farm labour decreases and boosting up productivity and efficiency for a better quality product (Giroud et al., 2016). Despite the difficulties and restrictions, the market for insect business has been growing with the rise of many firms and start-ups in Europe (Ynsect, Protix, Mutatec, and Hermetia Baruth GMBH) and the rest of the world (as Entofood, Agriprotein, Enviroflight, Enterra) (Arru et al., 2019). It is important to notice that insect rearing is practicable in developing countries since the only requirements for its development are a low-tech and low capital investment in order to achieve secure and quality products (Wang and Shelomi, 2017).

1. 6. 2. Insects nutritional value

The insects' nutritional profile is very difficult to establish once it relies on the developmental state (fat content can be higher in larval and pupal stages than at the adult stage), on the sort of feed composition they have in the wild or is provided with (vegetables, grains, or waste) and on treatment (drying procedures and deffating techniques) (Barroso et al., 2014; Gasco et al., 2018; Mariod, 2020).

Insects have a high protein content and can convert protein from diets to body mass levels in higher levels than poultry (accrue 33% compared to 22–45% in yellow mealworms and 43–55% in black soldier fly larvae) (Al-Qazzaz and Ismail, 2016; Henry et al., 2018; Rumpold and Schlüter, 2013; Huis and Oonincx, 2017). They are a rich source of lipids, 10-50%, and proteins with high biological value, 9-60%; due to the high-fat values, processing diets to defatting meals with solvents as petroleum ether or mechanical pressure is quite common in the feed industry once they can raise protein values to 70% and also, increase nutrient's accessibility (Basto et al., 2020; Choi et al., 2017; Henry et al., 2018). Table 2 provides the nutritional values of the most studied insect species for food and feed (black soldier fly, common housefly, and yellow mealworm).

Table 2. Comparison of chemical constituents (% DM), essential amino acids (g 16 g $^{-1}$ nitrogen), and fatty acid profile (% fatty acid) between some insects' species. Adapted from: Basto et al. (2020); Makkar et al. (2014).

	Black soldier fly	**Common housefly**	**Yellow mealworm**
Crude protein	41.1-43.6	42.3-60.4	47.2-60.3
Crude fat	15.0-49.0	9.0-26.0	29.4-43.1
Gross energy (mJ kg $^{-1}$ DM)	21.9-22.1	20.0-24.4	26.4-27.3
Ash	7.8-28.4	6.2-17.3	1.0-4.5
Calcium (g kg $^{-1}$ DM)	5.0-8.6	0.3-0.8	0.3-6.2
Phosphorus (mg kg $^{-1}$ DM)	6.4-15.0	9.7-24.0	4.4-14.2
Essential amino acids			
Arginine	5.3-6.1	3.7-5.8	3.8-5.6
Histidine	2.3-4.5	1.0-3.6	3.2-3.6
Lysine	6.0-8.0	5.0-8.2	4.6-6.1
Threonine	1.3-4.8	2.0-4.1	3.5-4.4
Isoleucine	4.7-5.6	2.3-3.7	4.1-5.0
Leucine	7.1-8.4	4.5-6.4	7.4-10.6
Valine	6.4-9.1	1.3-4.9	5.5-6.6
Methionine	1.7-2.4	1.3-3.7	1.3-2.0
Phenylalanine	4.6-5.6	3.7-5.9	3.5-4.3
Fatty acids			
Lauric acid (C12:0)	21.4	-	0.0-1.0
Myristic acid (C14:0)	2.9	4.1-6.8	2.3-6.4
Palmitic acid (C16:0)	16.1	26.7-38.0	16.1-28.7
Stearic acid (C18:0)	5.7	2.3-4.4	2.3-3.1
Palmitoleic acid (C16:1)	-	6.1-25.9	2.8-6.1
Oleic acid (C18:1)	32.1	21.8-27.7	27.7-43.3
Linoleic acid (C18:2)	15.5-24.0	16.4-23.1	23.1-31.0
Linolenic acid (C18:3)	0.2	2.0	1.1-1.4

Insects can provide high levels of essential and non-essential amino acids fulfilling the World Health Organization (WHO) guidelines, especially when it comes to lysine, methionine and leucine that are the most limiting amino acids in other sources as vegetable protein alternatives. Vitamins A, B_{1-12}, C, D, E and K also appears in the right values to fulfil human nutritional requirements (Al-Qazzaz and Ismail, 2016; FAO, 2013; Sánchez-Muros et al., 2014; Tang et al., 2019). Micronutrients are presented in high amounts of potassium, iron, magnesium, selenium and calcium values (Mancini et al., 2018; Mariod, 2020; Nogales-Mérida et al., 2019; Rumpold, and Schlüter, 2013).

Regarding fatty acids, there may be a disadvantage in the use of insects for fish feeding. Fat fluctuates from 7g to 77 g/100 g (dry weight), with a higher concentration in the larvae than the adults, but with a lack of EPA and DHA values when compared to FM, decreasing the n-3/n-6 ratio, especially the n-3 values. The highest values of SFA in insects are related to palmitic acid (80%) followed by monosaturated fatty acids (MUFA) represented by oleic acid, and finally, PUFAs mainly linoleic acid (Al-Qazzaz, and Ismail, 2016; Barroso et al., 2014; Gasco et al., 2018; Tang et al., 2019). This may lead to nutritional deficiencies that will affect final product quality concerning muscle properties and human nutritional necessities (Llagostera et al., 2019). However, great ductility of lipid content of some insects enables the increase of n-3 values when rearing larvae in fish by-products, such as fish waste, or seaweed (which in this case must be used carefully due to their levels of heavy metals and arsenic), leading to a 4% increase of PUFA, thus, lipid quality can be managed when a suitable feedstuff is designed to balance insect fatty acid content (Barroso et al., 2014; Cardinaletti et al., 2019; Gasco et al., 2018).

There is little information regarding the impact of chitin and antimicrobial peptides present in insects and that can affect fish health. Chitin is a polysaccharide part of insect exoskeleton, it is not digestible by humans (and is poorly digested by animals), turning it into an anti-nutritional factor. The main concern in human health is the risk of causing allergies, asthma, or inflammations in the human system (Al-Qazzaz and Ismail, 2016; Llagostera et al., 2019; Mariod, 2020). Despite all chitin problems, some authors allege that chitin can perform as a prebiotic and strengthen the intestinal microbiota with their immune stimulation (Sogari et al., 2019). Also, it can provide antifungal and antimicrobial traits, which could lead to improvements in fish immune status. As chitin, other components as lauric acid and antimicrobial peptides have bioactive traits that strengthen gut health, mainly against Gram-positive and negative bacteria (Llagostera et al., 2019; Gasco et al., 2018; Sogari et al., 2019).

Various insects' species were already tested in different trials with fish species as protein or lipid sources to replace dietary FM. Table 3 illustrates some insects that were already used in the diets of different fish species and the main results obtained. Later on, a focus will be placed on the insect used in this study (*Tenebrio molitor*).

Table 3. Insect species used in aquaculture trials and their main results.

Insect	Life stage	Applied Species	Inclusion levels (%)	Main results	Reference
Hermetia illucens	Larva	*Oncorhynchus mykiss*	0, 22, 44%	Proximate composition was not affected; A significant increment in SFA percentage.	(Mancini et al., 2018)
	Defatted Larva	*Salmo salar*	0, 5, 10, 15%	ADC* of protein, lipid, amino acid, and fatty acids was not affected; Increase of FI** and daily growth; FCR and SCR were not affected; The values of EPA and DHA decreased.	(Belghit et al., 2019)
	Larva	*Dicentrarchus labrax*	0, 10, 14, 20%	Growth performance was not affected at 50% of FM substitution (20% inclusion); The whole-body composition was not affected.	(Abdel-Tawwab et al., 2020)
	Pre-pupae		0, 7, 13, 20%	No differences among groups in growth performance or feed utilization; Plasma metabolic profiles remained unaffected; The ADC of arginine, histidine, and valine were higher in insect diets.	(Magalhães et al., 2017)
	Dried pre-pupae	*Sparus aurata*	0, 10, 20, 30%	Similar values of FCR, PER, and protein retention for fish fed with diets containing 10%, 20%, and 30% of FM substitutions.	(Karapanagiotidis et al., 2014)
Musca domestica	Dried maggot	*Oreochromis niloticus*	0, 11, 22, 33, 43%	Growth performance and ingredient utilization not affected in a replacement up to 270 g kg^{-1}; No significant influence on fillet proximate composition (DM, CP, CL, ash, and gross energy).	(Wang et al., 2017)
	Dried pupae	*Oncorhynchus mykiss*	0, 9%	FCR was not affected at 15% of substitutions; Fatty acid profiles suggested that fly larvae grown on manure do not have significant amounts of long-chain unsaturated fatty acids.	(St-Hilaire et al., 2007)

Tenebrio molitor	Defatted larva	*Oncorhynchus mykiss*	0, 5, 8, 15, 25%	Final body weight increased with the increasing levels of insects' inclusion; Improvement of growth rate, FCR, PR***; The whole-body composition was not affected; Protein, phosphorus, and energy retention significantly increased.	(Rema et al., 2019)
		Dicentrarchus labrax	0, 20%	Defatted TM showed the highest ADC for essential amino acids, energy, and phosphorous.	(Basto et al., 2020)
	Full-fat larva		0, 25, 50%	In whole-body composition, CP, and ether extract were not significantly influenced by the use of TM; ADC of the fish fed TM was higher than CTRL (92.31 vs 89.97, respectively).	(Gasco et al., 2016)
		Sparus aurata	0, 25, 50%	Substitution at 50% showed a higher final weight, SGR, weight gain, PER, and a lower FCR; No significant differences have been found in morphometric and commodity-related characteristics.	(Piccolo et al., 2017)
		Pagellus bogaraveo	0, 12, 23%	Daily intake ratio, FCR, and SGR were not affected by different diets, like slaughter traits and carcass yield; Different diets did not affect the colour of the skin dorsal region; In colour of the fillet epaxial region, yellowness, and chroma were higher when TM was added to the diets.	(Iaconisi et al., 2017)

*ADC, apparent digestibility coefficient; **FI, feed intake; ***PR, protein retention;

1. 7. Yellow mealworm (*Tenebrio molitor*)

Tenebrio molitor (TM) (Figure 5), commonly known as yellow mealworm, is a worldwide distributed Coleoptera and is an up-and-coming aspirant as a new protein source for FM substitution in fish feeds and also a novel source for human consumption in Europe (Arru et al., 2019; Llagostera et al., 2019; Iaconisi et al., 2017a; Paul et al., 2017).

Figure 6. The adult stage of *Tenebrio molitor*. Photo credits and copyright: https://www.lifeonwhite.com/

TM is already produced industrially and commercially to feed pets and zoo animals, mainly reptiles, small mammals, amphibians, and birds, but also as fishing baits and for human consumption (Belforti et al., 2015; Grau et al., 2017). They are easy to breed and feed both in larval and pupal stages and are rich in protein and lipid, thus being easy to mass-produce and therefore suitable for aquaculture; China is already doing that in the past years (Arru et al., 2019; Belforti et al., 2015; Llagostera et al., 2019; Iaconisi et al., 2017a). The main advantage of the larvae stage is that they're raised on low-nutritive plant waste products as dried fruit, vegetable, and cereal residues in various combinations, so its production is not exactly expensive (Henry et al., 2018; Tinder et al., 2017). Also, chitin content in the larval stage is considerably lower when compared with adults. Costa et al. (2020) and Janssen et al., (2017) measured the chitin content of *Tenebrio molitor* larvae and obtained 5.3% and a range of 3.8-6.8% DM of chitin, respectively. Finke (2007) registered 7.3% DM of chitin content in adult mealworm.

At the nutritional level, TM is a rich source of protein that may vary between 47 to 60% and up to 70% when defatted, suitable for aquaculture diets (Llagostera et al., 2019; Henry et al. 2018; Ng, 2001). Concerning amino acids, they are rich in isoleucine, leucine, and lysine (Arru et al., 2019). TM is also rich in energy, phosphorus, and many other trace nutrients as zinc, selenium, riboflavin, biotin, pantothenic acid, and folic acid (Arru et al., 2019; Klasingph, Lopez et al., 2000). Their lipid content varies between 31 to 43%, and they don’t have 20:3 n-6 and 20:4 n-6 fatty acids, which can lead to some nutritional deficiencies (Al-Qazzaz and Ismail, 2016; Llagostera et al., 2019; Henry et al., 2018). The highest values of fatty acids are related to oleic acid (37.7% fatty acid), linoleic (27.4% fatty acid), and palmitic acid (21.1% fatty acid) (Makkar et al., 2014).

1. 8. Aim species: European seabass (*Dicentrarchus labrax*)

European seabass (*Dicentrarchus labrax*, Linnaeus, 1758) is a euryhaline marine teleost species, a member of the Moronidae family and is widely distributed in the Mediterranean and Atlantic coast from the English Channel to Senegal (Abbate et al., 2012; Eroldoğan et al., 2004). Living near the coasts and estuaries that are rich in microorganisms, seabass has been farmed through aquaculture for a long time now (Tacon et al., 2011). The preference for this fish is due to its organoleptic properties like taste and aroma, acceptable price, nutritional value, and overall quality (Ayala et al., 2005; Fuentes et al., 2010). Moreover, seabass is an excellent source of protein and lipids for human consumption (Delgado et al., 1994). The commercial size of farmed fish is < 400 g for portion-size and can reach this size in 12 to 18 months depending on diets or between 800 g and 1 kg for larger fish (EUMOFA, 2018).

1. 8. 1. Nutritional requirements

European seabass, as a carnivorous fish species, requires very high protein values, around 50% of the diet to maximize growth in juveniles (Hidalgo and Alliot, 1988; Peres and Oliva-Teles, 1999). For seabass juveniles, protein requirements could be between 43 to 48% of the diet when given the adequate dietary digestible energy (DE) levels (Peres and Oliva-Teles, 1999). To overcome the need for large quantities of protein, a balanced ratio of digestible protein (DP)/DE is important. For seabass, this ratio should be 19 mg/kJ in diets with at least 21 MJ/kg DE, with the major portion of non-protein energy being supplied by lipid supplementation that will cause a protein-sparing effect, increasing the DE and reducing nitrogen losses (Dias et al., 1998; Lupatsch et al., 2001; Peres and Oliva-Teles, 1999).

An adequate level of protein in the diet is necessary mainly to ensure the amount of essential amino acids (EAA) needed for fish growth (Mansano et al., 2020). For European seabass, as for other finfish, the essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Wilson, 1986). Their requirements for European seabass are presented in Table 5. More than EAA needs, the right balance between non-essential amino acids (NEAA) and EAA is needed to ensure high growth performance and effective protein and energy utilization. In seabass juveniles, the optimal ratios of EAA:NEAA are 50:50 and 60:40 (Peres and Oliva-Teles, 2007).

Table 4. Available values of dose-response indispensable amino acids requirement estimates (g/16gN) European seabass. Retrieved from: (Tibaldi, and Kaushik, 2005).

Amico acid	**(g/16 g N)**
Arginine	3.9-4.6
Lysine	4.8
Methionine + Cysteine	4.0
Threonine	2.6
Tryptophan	0.5-0.7

Commercial feeds for seabass are highly energetic, and optimal levels of lipids can improve high-quality fats, growth, feed conversion, and protein utilization (Izquierdo et al., 2003). To maximize these effects, levels between 18-20% of fatty acids are recommended (Campos et al., 2019). To measure lipid retention in European seabass it's important to keep in mind that the liver is the primary site of lipid storage, contrary to salmonids, which store their lipids in the perivisceral adipose tissue first (Dias et al., 2005). As European seabass is a carnivorous marine fish, it has a limited ability to elongate and de-saturate C18 fatty acids to EPA and DHA, and these fatty acids must be supplemented in the diet (NRC, 2011). As regards essential fatty acids for juvenile European seabass, levels of 1% of n-3 PUFA are estimated, meaning 10 g kg^{-1} diet, and 2-3 g kg^{-1} of total lipids (Coutteau et al., 1996; Kousoulaki et al., 2015).

Regarding carbohydrates, European seabass has a limited capacity to tolerate high percentages (> 30% dietary starch) since this commodity seems to reduce growth and feed utilization (Enes et al., 2011).

1. 9. Aim of this study

This study hypothesizes that if an insect meal is included in a well-balanced diet, FM can be replaced in diets for European seabass without affecting flesh nutritional and sensory quality, and consumers acceptance. Thus, this study aimed to assess the effects of increasingly replacing FM (0%, 50% and 100%) by defatted *Tenebrio mealworm* larvae meal on the European seabass whole body gain and retention of fatty acids, flesh fatty acids profile, flesh instrumental colour and texture and flesh sensory profile and global acceptance by consumers.

2. Material and Methods

2. 1. Ingredients and experimental diets

Based on the known nutritional requirements of European seabass (NRC, and National Research Council, 2011), three experimental diets were formulated to be isoproteic (47% DM), isolipidic (20% DM), and isoenergetic (24 kJ/g DM). A FM based diet was used as a control diet (CTRL) and compared with two other experimental diets with 50% (TM50) and 100% (TM100) of FM replacement by defatted *Tenebrio molitor* larvae meal (TM). For these replacement levels, the inclusion levels of TM tested in diets were 0% in CTRL, 20% in TM50 and 40% in TM100. All experimental diets were extruded by SPAROS, Lda. (Portugal). The formulation and proximate composition of diets are available in Table 6 and the fatty acid profile of the ingredient and diets are available in Table 7.

2. 2. Fish, rearing conditions, and operative protocol

The present study was performed by accredited scientists in laboratory animal science by the Portuguese Veterinary Authority DGAV-Portugal and conducted according to the Directive 2010/63/EU of the European Parliament and the Council on the protection of animals for scientific purposes.

The growth trial was conducted at the experimental facilities of CIIMAR, Matosinhos, Porto, Portugal, and the European seabass juveniles were supplied by a commercial fish farm Acuinuga – Acuicultura y Nutrición de Galicia, S.L. (Coruña, Spain). To adapt to the experimental conditions, fish were kept in quarantine for 2 weeks and fed a commercial diet (AQUASOJA, Sorgal S.A., Portugal – 50% crude protein, 20% crude fat DM basis). After acclimatization, 12 homogeneous groups of 15 fish (mean body weight 68.64 ± 5.24 g) were randomly distributed by 160 L fiberglass tanks in a saltwater closed recirculation system. Fish were adapted to the new conditions for 4 weeks. The system was daily maintained and controlled for total ammonium, nitrite, nitrate, and pH levels to ensure levels within the recommended ranges for marine species, and dissolved oxygen levels were kept above 90% ± 1 saturation. Also, water temperature (22 ± 1 °C), salinity (35 ± 0.5 ‰), and a photoperiod of 12h light/12 h dark and water flow rate of 6 L min^{-1} were maintained. Fish were fed using temporized automatic feeders (initially the amount of diet provided was 1.5% of total body weight/day). The amount of feed was adjusted by 5% based on the presence or absence of uneaten food at the bottom of the tanks over two days. Each diet was distributed to quadruplicate groups of fish for 120 days.

Table 5. Ingredients and proximate composition of the experimental diets.

	TM*d*	**CTRL**	**TM50**	**TM100**
Ingredients (%)				
Fish meal Super Prime [a]		40.0	20.0	-
Tenebrio meal [b]		-	20.5	40.4
Soy protein concentrate [c]		10.5	10.5	10.5
Soybean meal [d]		13.0	13.0	13.0
Rapeseed meal 48 [e]		5.0	5.0	5.0
Wheat meal [f]		16.2	15.2	14.3
Fish oil [g]		14.0	13.3	12.5
Vitamins and minerals premix [h]		1.0	1.0	1.0
Vitamin C		0.1	0.1	0.1
Vitamin E		0.1	0.1	0.1
Monocalcium phosphate		-	1.2	2.5
L-Lysine		-	-	0.2
L-Threonine		-	-	0.2
L-Tryptophan		-	-	0.1
DL-Methionine		0.1	0.2	0.3
Chemical composition (%DM)				
Dry matter	97.8	93.1	92.6	92.5
Crude protein	71.0	46.9	47.3	47.2
Crude fat	12.5	19.7	19.8	19.0
Gross energy (kJ g^{-1} DM)	24.3	23.2	23.5	24.0
Phosphorus	0.8	1.2	1.2	1.0
Ash	4.8	10.2	8.1	6.3

The abbreviations for the experimental diets stand for: CTRL – control diet; TM50 and TM100 – diets with 50 and 100% fish meal replacement by *Tenebrio molitor* larvae meal; TMd – defatted *Tenebrio molitor* larvae meal;

[a] Peruvian fish meal super-prime: 71.0% crude protein (CP), 11.0% crude fat (CF), Exalmar, Peru.

[b] *Defatted Tenebrio* molitor larvae meal: 71% CP, 13% CF.

[c] Soy protein concentrate: 65% CP, 0.7% CF, ADM Animal Nutrition, The Netherlands.

[d] Soybean meal 48: Dehulled solvent-extracted soybean meal: 47.7% CP, 2.2% CF, Cargill, Spain.

[e] Rapeseed meal: 36% CP, 2.7% CF, PREMIX Lda., Portugal;

[f] Wheat meal: 10.2% CP, 1.2% CF, Casa Lanchinha, Portugal;

[g] Sardine oil, Sopropêche, France.

[h] Vitamin and mineral premix: InVivo,

Table 6. Fatty acid profile of the experimental diets.

	Experimental diets			
	TM*d*	**CTRL**	**TM50**	**TM100**
Fatty acids (g 100 g^{-1} total fatty acids)				
SFA				
C14:0	2.15	5.51	5.49	5.59
C16:0	16.18	14.80	15.83	17.55
C18:0	4.48	3.44	3.69	4.11
MUFA				
C18:1n9c	34.20	16.06	16.60	16.76
C18:1n7	0.39	3.14	2.75	2.30
C20:1n9	0.13	4.39	3.87	3.22
C22:1n9	0.06	0.20	0.13	0.10
PUFA				
C18:2n6c	38.29	6.40	10.37	14.19
C18:3n3	1.32	2.12	1.71	1.31
C18:4n3	0.03	2.81	2.68	2.47
C20:5n3		8.90	7.87	6.94
C22:5n3		1.54	1.31	1.05
C22:6n3		10.43	8.90	7.34
EPA + DHA [1]	0.00	19.33	16.77	14.28
Σ SFA [2]	23.88	26.83	27.96	30.17
Σ MUFA [3]	36.36	30.32	29.42	27.94
Σ PUFA [4]	39.76	42.84	42.62	41.89
Σ n-3 [5]	1.35	26.67	23.26	19.81
Σ n-6 [6]	38.32	15.76	19.05	21.87
Σ n-3/Σ n-6 [7]	0.04	1.69	1.22	0.91
Σ n-6/Σ n-3 [8]	28.36	0.59	0.82	1.10

[1] EPA + DHA = eicosapentaenoic acid (20:5n-3) + docosahexaenoic acid (22:6n-3);

[2] ∑ SFA, sum of saturated fatty acids, includes 12:0, 13:0, 14:0 15:0, 16:0 17:0, 18:0, 20:0, 21:0;

[3] ∑ MUFA, sum of monounsaturated fatty acids, includes 16:1n-9, 14:1n-5, 17:1n-7, 18:1n-9, 18:1n7, 22:1n-11;

[4] ∑ PUFA, sum of polyunsaturated fatty acids, includes 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-6, 18:2n-4, 18:3n-3, 18:3n-6, 18:4n-1, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 21:5n-3, 22:5n-3, 22:5n-6, 22:6n-3;

[5] ∑ PUFAn-6, sum of n-6 PUFA, includes 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:5n-6;

[6] ∑ PUFAn-3, sum of n-3 PUFA, includes 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3;

[7] Σ n-3/Σ n-6, ratio between the sum of n-3 PUFA and n-6 PUFA;

[8] Σ n-6/Σ n-3, ratio between the sum of n-6 PUFA and n-3 PUFA;

2. 3. Sampling procedures

Before initial sampling, fish fasted for 24 hours and 10 fish were sampled and pooled from the initial stock and stored at -20 °C for subsequent whole-body composition analysis. Also, 30g of each experimental diet was frozen and stored at -80 °C to analyse chemical composition. At the end of 16 weeks of growth trial, fish were fasted for 48 hours and were sacrificed by anaesthetic overdose (2-Phenoxyethanol, 700 µl L^{-1}), individually weighed (g), and measured (total length, cm). Four fish/tank (16 fish/treatment) were collected to evaluate proximal composition, placed in a plastic bag, and stored at -20°C until analysis. Three fish/ tank were collected to evaluate 1) the viscerosomatic and hepatosomatic indexes were liver and viscera were weight (g); 2) the instrumental colour of skin; and 3) the instrumental colour, texture and nutritional quality of muscle. A representative sample of muscle (1 x 1 cm) was collected without skin from the right dorsal fillet and placed in ice for further instrumental colour and texture analyses. Two representative samples of the left fillet without skin were sampled, immediately frozen in liquid nitrogen, and stored at -80 ºC until dry matter, total lipid content, and fatty acid profile analysis. Five fish/tank were euthanized in ice baths for consumer's global acceptance evaluation.

2. 4. Chemical analyses

Proximate composition

Proximate composition analyses were performed according to AOAC (2000) methods. All samples were analysed for dry matter, in an oven at 105 °C for 24h; ash by combustion in a muffle furnace, incinerated at 500 °C for 5 h (Nabertherm L9/11/B170, Bremen, Germany); crude protein (N × 6.25) using a Leco nitrogen analyser (Model FP 528; Leco Corporation, St. Joseph, USA); crude fat by petroleum ether extraction using a Soxtec extractor (Model ST 2055 Soxtec™; FOSS, Hillerod, Denmark); phosphorus content by digestion at 230 °C in a Kjeldatherm block digestion unit followed by digestion at 75 °C in a water bath and absorbance determination at 820 nm (adapted from AFNOR V 04-406) and gross energy was determined in an adiabatic bomb calorimeter (Model Werke C2000, IKA, Staufen, Germany.

Total lipid extraction and fatty acid profile

Total lipids of carcass and muscle were extracted and quantified gravimetrically with the method described by Folch (1957) but using an adapted version with dichloromethane:methanol (2:1) instead of chloroform:methanol (2:1).

The fatty acids in the lipid extracts were transesterified by acid methylation to fatty acid methyl esters (FAME) (Campos et al., 2017). The FAME in the experimental diets were obtained using the same procedure but by direct transesterification. Tricosanoic acid (23:0) was added as an internal standard in the samples for later calculation of fatty acid concentration; FAME were recovered in 1 ml of n-Hexane.

The FAME separation was performed in a gas chromatography system with a gas chromatograph, where the drag gas used was helium, (Shimadzu GC-2010 Plus, Tokyo, Japan) coupled with an AOC-20i auto-injector (Shimadzu, Tokyo, Japan) and a flame ionisation detector (Shimadzu, Tokyo, Japan). A CP-Sil 88 silica capillary column (50.0 m × 0.25 mm internal diameter and 0.20 μm film thickness) (Middelburg, Netherlands) was used. The temperature programme used was as follows: 120 °C for 5 min; increase from 2 °C/min to 160 °C for 15 min; and increase from 2 °C/min to 220 °C for 10 min. The injector and detector temperatures were 250 and 270 °C respectively. A division ratio of 1:50 was used and the injection volume was 1.0 μL (105). FAME was identified by comparison with a standard mixture (FAME 37, Supelco, Bellefonte, PA, USA). Each FAME was expressed as a relative percentage of the total FAME area represented on the chromatogram: A(%) = (area A ×100) / (sum of all peak areas), according to Reis et al. (2014) . The amount of fatty acids was calculated using the internal standard (C:23) as reference: Fatty acid (AG) composition in muscle (mg/g fresh weight) = [(area AG × TRFAG × mass C23) / area C23 × sample mass × TRFC23)] × 100, according to Joseph and Ackman (1992).

2. 5. Colour and texture analysis

Instrumental colour analysis

Skin and muscle colour measurements were performed with a CR-400 colourimeter (Figure 7) (Konica Minolta) with an aperture of 8 mm, at standard illuminate D65 using the CIE *L**, *a** and *b**. This compares the reflectance of light from an object (fish fillet and skin) and for that, the apparatus was calibrated with a white plate reference standard (Minolta Co., Ltd., Osaka, Japan).

Figure 7. Skin colour analysis with CR-400 colourimeter.

The fillet replicates measurements were taken for each sample and averaged to determine the colour parameters, which were measured by applying the colourimeter onto the skin and muscle. After flashing, *L**, *a*,* and *b** reflected light values were recorded. *L** represents lightness (negative for blackness and positive for whiteness), *a**, or redness, indicates red/green chromaticity (negative for greenness and positive for redness), and *b*,* or yellowness, indicates yellow/blue chromaticity (negative for blueness and positive for yellowness). From *a** and *b** values were calculated the hue angle and the chroma according to Valente et al., (2015). Hue is the relationship between redness and yellowness and is an angular measurement of colour where 0° and 90° denote red and yellow hues, respectively, which is expressed as $H^o = \tan^{-1} b^* / a^*$. Chroma is expressed as $C^o = (a^{*2} + b^{*2})^{1/2}$ and gives information about the clarity and intensity of the colour. All these parameters are represented in Figure 8.

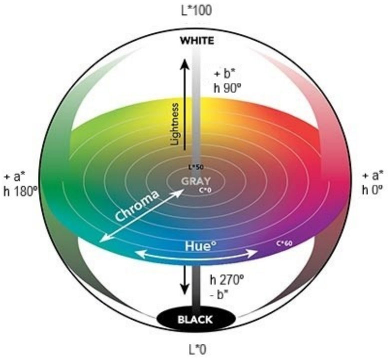

Figure 8. CIE *L* a* b** colour space diagram. Retrieved from: https://www.xrite.com/

Texture profile analysis

The fillet was collected following the longitudinal orientation of the muscular fibres and texture was analysed using a Texture Analyzer Model Instron 4301 (Instron Engineering, Canton, MA, USA), equipped with a load cell of 0.005 N and a 2.0 mm diameter probe (Figure 9). Texture profile analysis was obtained by double compression (constant speed and penetration depth of 1 mm s^{-1} and 4.0 mm, respectively) on the maximum thickness part of each raw fillet. The parameters determined were hardness, cohesiveness, springiness, gumminess, resilience, and chewiness. Hardness corresponds to the maximum force required to compress the fillet. Adhesiveness corresponds to the energy needed to overcome the attractive forces of the fillet. Cohesiveness is the extent to which the fillet could be deformed before rupture [(A3 + A4)/(A1 + A2), where A1 + A2 was the total energy required for the 1st compression and A3 + A4 was the total energy required for the 2nd compression]. Springiness is the ability of fillet to recover its original form after the deforming force is removed (L2/L1, where L1 was the lengthening of the 1st compression and L2 was the lengthening of the 2nd compression). Resilience shows how well a product fights to regain its original position (A2/A1, where A1 was the total energy required for compression of the 1st compression and A2 was the total energy required for decompression of the 1st compression). Finally, chewiness is the work needed to chew a solid sample to a steady state of swallowing.

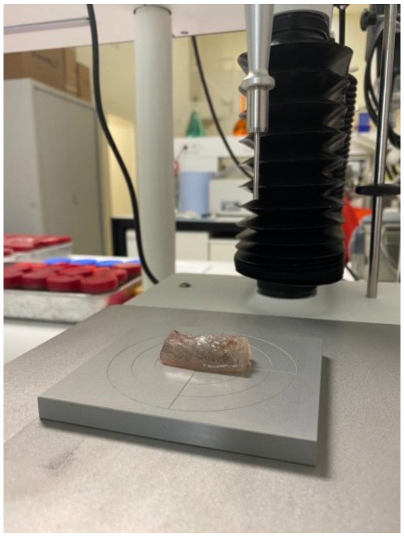

Figure 9. Texture instrumental analysis with Texture Analyzer Model Instron.

2. 6. Sensory Analysis

The sensory study was performed by Sense Test (Sociedade de Estudos de Análise Sensorial a Produtos Alimentares, Lda.) aiming to assess the sensory perception of different seabass fed with the experimental diets. The study was divided into two steps: firstly, the global acceptance of seabass samples was evaluated by the consumer and secondly, the panellists' added free comments, concerning the relationship between the samples and the negative and positive aspects. Before sensory analysis, all fish were cleaned with the removal of viscera and scales. After that, fish were cut into three slices, anterior (close to the head), central and posterior (close to the caudal fin) (Figure 10). Seabass samples were then wrapped in microperforated aluminium foil and steamed for 12 minutes at 100 ºC in an industrial convector oven (Rational) preheated to 100 ºC. To each consumer the 3 samples were presented, monadically and sequentially, always evaluating posts of the same position. The presentation order was balanced among consumers. The global acceptance was evaluated on 9 points hedonic scale. After that, each consumer made a free comment to each sample, considering the main negative and positive points. The comments were related to appearance, odour, texture and taste (appears in the results, Table 14). Due to the sample number limitation (29 fish per treatment), each reference was evaluated by 57 participants. The average age was 46 years old, with a minimum age of 27 years and a maximum of 64 years. 63% of the participants were women.

Figure 10. Fish samples for panellist's acceptance evaluation.

2. 7. Statistical Analysis

Data were initially tested for normally and homogeneity of variances by Levene’s tests and transformed whenever required before being submitted to a one-way ANOVA. When this test showed significances, individual means were compared using HSD Tukey’s multiple range test. A non-parametric test (Kruskal-Wallis H-test) was applied when these assumptions were not achieved after transformation. Significant differences were considered for a *p*-value < 0.05. The software used for statistical analysis was SPSS (IBM-SPSS Statistics v.25.0, SPSS Inc., Chicago, IL, USA) and the one used for graphic representation was GraphPad (GraphPad Software Inc).

2. 8. Calculations

ABW (Average Body Weight) (g) = (initial weight + final weight) / 2 (g)

SGR (Specific growth rate) = (LN final weight - LN initial weight) / number of days experience * 100 (g/100 g/day)

VFI (Voluntary Feed Intake) = 100 x Feed consumption (g/fish) / g ABW / number of experience days (g/100 g/day)

FCR (Feed Conversion Ratio) = Feed consumption (g DM / fish) / (final weight (g) - initial weight (g))

K factor (Condition index) = 100 x final weight / final length$^{\wedge 3}$

HSI (Hepatosomatic index) = 100 x (liver weight / final fish weight) (%)

VSI (Viscerosomatic index) = 100 x (viscera weight / final fish weight) (%)

PER (Protein Efficiency Ratio) = weight gain (g) / Fish Ingested Protein (g) = (final weight - starting weight) (g) / Fish Ingested Protein (g)

Nutrient gain, energy and fatty acids (Nutrient gain, g / kg ABW / day) = (final weight (g) x% nutrient in the final composition in WW x%) - (initial weight (g) x% nutrient in the initial composition in WW x%) / ABW (kg) / number of days of experience = G nutrients (g) / ABW (kg) / days of experience

Retention of nutrients, energy, and fatty acids (% of intake) = 100 × [(final weight × final nutrient content of carcass - initial weight × initial nutrient content of carcass) / (intake × nutritional content of diet (% DM)];

3. Results

3. 1. Growth performance

Overall, all the experimental diets were well accepted by fish that almost quadruplicated their initial body weight. No mortality occurred during the entire trial. The growth performance results of the fish fed by the different experimental diets are presented in Table 7. Fish reached a final body weight of 267g and no significant differences were observed among dietary treatments. Regarding the voluntary feed intake (VFI), results indicated that fish fed CTRL had the highest values, 17.29 ± 0.23, and TM50 the lowest ones, 15.79 ± 0.72. FCR and PER varied significantly between diets (Figure 10). FCR had the lowest values in TM50 (1.00 ± 0.03) whilst PER had the highest values in fish fed with TM50 (0.14 ± 0.005).

Concerning the whole-body composition, CF was the only parameter that presented significant differences amongst diets, increasing their values with the highest inclusion percentage of insects (TM100).

3. 2 Total lipids and fatty acids profile

The whole-body lipid content and fatty acid profile of European seabass fed the experimental diets is presented in Table 8. Total lipids did not vary significantly among fish fed the different experimental diets (12-13%). Significant differences between diets were only observed in the sum of MUFA (p=0.035) and PUFA (p=0.015): fish fed TM100 had higher MUFA values than CTRL; in contrast, fish fed TM100 had lower PUFA values than CTRL (Figure 12).

Regarding fish muscle total lipids and fatty acid profile, values are presented in Table 9, with some significant differences in the majority of the fatty acids. Muscle lipid content remained unaffected by the inclusion of TM, in spite of a trend for increasing lipid deposition. Concerning SFA percentage, TM100 had the highest values, differing both from the CTRL and from the TM50. The MUFA and PUFA did not vary significantly among dietary treatments, although TM100 had the lowest values. However, it is important to highlight several differences present in the principal PUFA: fish fed TM100 had significantly lower values of EPA, 6.81 ± 0.22 g/100 g fatty acids, followed by TM50, 7.43 ± 0.14 g/100 g, and CTRL, which had the highest values, 8.23 ± 0.13 g/100 g (p=<0.001).

Table 7. Final growth performance and somatic indexes of European seabass.

	Experimental diets			
	CTRL	**TM50**	**TM100**	***p*-value**
Growth performance				
Initial body weight (g)	68.63 ± 5.16	68.65 ± 5.19	68.64 ± 5.05	0.909
Final body weight (g)	266.99 ± 34.16	267.54 ± 34.10	267.88 ± 33.67	0.937
Initial body length (cm)	18.88 ± 0.56	18.87 ± 0.6	18.81 ± 0.55	0.309
Final body length (cm)	27.92 ± 1.15	27.90 ± 1.13	27.90 ± 1.17	0.912
Condition factor (final K)	1.22 ± 0.10	1.23 ± 0.09	1.25 ± 0.08	0.098
Specific growth rate (SGR) (g/100 g/day)	1.21 ± 0.11	1.21 ± 0.13	1.22 ± 0.14	0.940
Voluntary feed intake (VFI) (g/100 g/day)	17.29 ± 0.23 ^{a}	15.79 ± 0.72 ^{b}	17.01 ± 0.94 ^{ab}	0.031
Feed conversion ratio (FCR)	1.10 ± 0.03 ^{a}	1.00 ± 0.03 ^{b}	1.07 ± 0.05 ^{a}	0.012
Protein efficiency ratio (PER)	0.13 ± 0.004 ^{b}	0.14 ± 0.005 ^{a}	0.13 ± 0.01 ^{b}	0.014
Viscerosomatic index (VSI) (%)	6.59 ± 1.17	6.71 ± 1.19	5.95 ± 0.92	0.467
Hepatosomatic index (HIS) (%)	2.03 ± 0.43	2.17 ± 0.43	2.08 ± 0.44	0.647
Whole-body composition (% WW)				
Moisture	62.91 ± 0.58	62.19 ± 0.21	62.15 ± 0.75	0.083
Crude Protein	18.21 ± 0.14	18.06 ± 0.39	17.94 ± 0.23	0.431
Crude Fat	15.77 ± 0.78 ^{b}	16.79 ± 0.36 ^{ab}	17.01 ± 0.57 ^{a}	0.036
Gross Energy (kJ g^{-1} DM)	10.01 ± 0.36	10.14 ± 0.11	10.23 ± 0.19	0.456
Phosphorus	0.59 ± 0.02	0.60 ± 0.02	0.62 ± 0.03	0.237

Values are presented as mean ± standard deviation (n=4). Values in the same row without a common superscript letter differ significantly ($p < 0.05$).

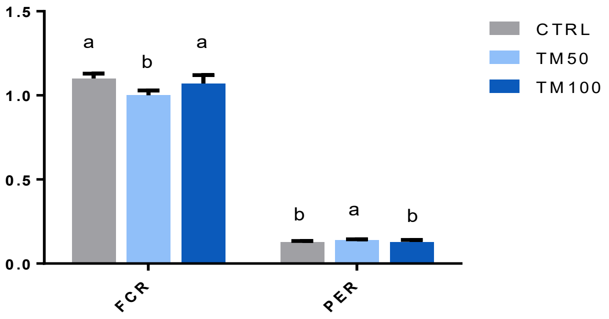

Figure 11. Differences in the feed conversion ratio (FCR) and protein efficiency ratio (PER) of European seabass fed with the experimental diets. Bars with a common superscript letter differ significantly ($p < 0.05$). Letters "a" refers to values statistically different and higher than values referred as "b". Letters "a" are against "b" but only within each group, FCR or PER.

Regarding DHA, the inclusion of TM resulted in a significant reduction of this fatty acid in the muscle. However, when a fillet portion of 100 g is considered, the absolute values of EPA + DHA remained above the recommended levels for human consumption (0.26 and 0.30 / 100 g of wet weight in TM100 and TM50, respectively) in all fish, and did not vary significantly among treatments (*p*=0.249).

Retention values are presented in Table 10 and differences are more evident in the sums of fatty acids, mainly between CTRL and TM50. The inclusion of TM resulted in a significant increase of SFA retention in TM50 compared to CTRL, 57.31 ± 7.46 vs 42.16 ± 3.74, respectively (*p*=0.015). MUFA had the highest retention values in fish fed TM, irrespectively of the inclusion level (p=0.005); the main fatty acid responsible for such result was the C18:1n9c that was highly retained particularly in fish fed TM50. PUFA retention was also promoted by the dietary inclusion of TM, with the retention of C18:4n3 being significantly higher in fish fed TM50 than in those fed the CTRL (*p*=0.032).

In Table 11 is presented the nutrient gain of some analysed fatty acids and the major significant differences are in the sum of all SFA (*p*=0.029*)* and MUFA (*p*=0.025), with TM100 showing the highest fatty acids gain. The inclusion of TM significantly increased the gain of C16:00 and C18:0 in TM100. Likewise, the gain of oleic (221.59 ± 31.62 mg/kg ABW /day) and linoleic acids (102.30 ± 26.43 mg/kg ABW /day) was significantly higher in fish fed TM100 when compared to CTRL (108.82 ± 84.65 and 57.13 ± 15.69 mg/kg ABW /day, respectively) (Figure 13). The gain of total PUFA remained unaffected by the dietary treatments, in spite of a trend for an increased gain of EPA and DHA.

Table 8. Fish whole-body lipid content (% wet weight, WW) and fatty acid composition (g 100 g^{-1} total fatty acids) of the European seabass.

	Experimental diets				*p*-value
	INITIAL	**CTRL**	**TM50**	**TM100**	
Total lipids (% WW)	7.68	12.28 ± 4.39	12.85 ± 2.25	13.12 ± 1.67	0.94
Fatty acids (g 100 g^{-1} total fatty acids)					
C14:0	3.76 ± 0.01	4.50 ± 0.54	4.12 ± 0.08	4.05 ± 0.05	0.136
C16:0	19.88 ± 0.10	20.04 ± 2.17	19.55 ± 0.16	19.82 ± 0.52	0.874
C18:0	4.17 ± 0.05	3.56 ± 0.32	3.38 ± 0.03	3.48 ± 0.12	0.486
Σ SFA [1]	29.78 ± 0.08	30.27 ± 3.29	28.96 ± 0.19	29.24 ± 0.52	0.614
C18:1n9c	25.35 ± 0.00	17.79 ± 10.03	24.05 ± 0.61	24.75 ± 0.96	0.221
C18:1n7	3.03 ± 0.01	3.25 ± 0.58	2.88 ± 0.07	2.73 ± 0.33	0.201
C20:1n9	2.17 ± 0.03	3.60 ± 0.66	3.13 ± 0.05	2.89 ± 0.42	0.726
Σ MUFA [2]	38.42 ± 0.18	32.12 ± 7.87^{b}	37.14 ± 0.35^{a}	37.05 ± 0.31^{a}	0.035
C18:2n6c	11.58 ± 0.02	9.51 ± 2.60	10.34 ± 0.24	11.57 ± 2.61	0.423
C18:3n3	1.43 ± 0.07	1.77 ± 0.38	1.50 ± 0.03	1.38 ± 0.26	0.166
C18:4n3	0.31 ± 0.09	2.15 ± 0.33	1.93 ± 0.02	1.83 ± 0.14	0.134
C20:5n3	6.61 ± 0.03	7.18 ± 1.30	6.06 ± 0.14	5.72 ± 0.66	0.081
C22:5n3	1.19 ± 0.04	1.43 ± 0.31	1.18 ± 0.02	1.10 ± 0.18	0.109
C22:6n3	7.55 ± 0.03	9.24 ± 2.10	7.65 ± 0.21	7.11 ± 1.20	0.136
Σ PUFA [3]	31.79 ± 0.10	37.61 ± 4.63^{a}	33.90 ± 0.47^{b}	33.71 ± 0.48^{b}	0.015
Σ n-3 [4]	17.37 ± 0.13	22.33 ± 4.50	18.72 ± 0.32	17.57 ± 2.48	0.114
Σ n-6 [5]	13.84 ± 0.03	14.69 ± 2.25	14.66 ± 0.19	15.63 ± 2.22	0.703
Σ n-3/Σ n-6 [6]	1.26 ± 0.01	1.55 ± 0.38	1.28 ± 0.01	1.16 ± 0.37	0.239
Σ n-6/Σ n-3 [7]	0.80 ± 0.12	0.68 ± 0.22	0.78 ± 0.01	0.91 ± 0.22	0.254
Fatty acids (g/100 g WW)					
C20:5n3	0.25 ± 0.02	0.38 ± 0.07	0.52 ± 0.19	0.41 ± 0.08	0.713
C22:6n3	0.28 ± 0.02	0.49 ± 0.11	0.64 ± 0.22	0.50 ± 0.12	0.834
EPA + DHA [8]	0.53 ± 0.04	0.87 ± 0.18	1.16 ± 0.20	0.90 ± 0.20	0.793

[1] ∑ SFA, sum of saturated fatty acids, includes 12:0, 13:0, 14:0 15:0, 16:0 17:0, 18:0, 20:0, 21:0; [2] ∑ MUFA, sum of monounsaturated fatty acids, includes 16:1n-9, 14:1n-5, 17:1n-7, 18:1n-9, 18:1n7, 22:1n-11; [3] ∑ PUFA, sum of polyunsaturated fatty acids, includes 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-6, 18:2n-4, 18:3n-3, 18:3n-6, 18:4n-1, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 21:5n-3, 22:5n-3, 22:5n-6, 22:6n-3; [4] ∑ n-3, sum of n-3 PUFA, includes 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3; [5] n-6, sum of n-6 PUFA, includes 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:5n-6; [6] Σ n-3/Σ n-6, ratio between the sum of n-3 PUFA and n-6 PUFA;[7] Σ n-6/Σ n-3, ratio between the sum of n-6 PUFA and n-3 PUFA; [8] EPA + DHA = eicosapentaenoic acid (20:5n-3) + docosahexaenoic acid (22:6n-3); Values are presented as mean ± standard error of the mean (n = 4);Values in the same row with a common superscript letter differ significantly ($p < 0.05$).

Table 9. Muscle total lipid content (% wet weight, WW) and fatty acid composition (g 100 g^{-1} total fatty acids) of the European seabass fed the experimental diets.

	CTRL	TM50	TM100	*p*-value
Total lipids (% WW)	2.49 ± 0.26	2.68 ± 0.36	2.58 ± 0.38	0.870
Fatty acids (g 100 g-1 total fatty acids)				
C14:0	3.45 ± 0.10	3.32 ± 0.05	3.38 ± 0.07	0.096
C16:0	18.67 ± 0.10^{b}	19.11 ± 0.27^{b}	19.79 ± 0.25^{a}	< 0.001
C18:0	3.92 ± 0.08^{b}	4.11 ± 0.06^{a}	4.22 ± 0.08^{a}	0.001
Σ SFA [1]	28.11 ± 0.08^{b}	28.66 ± 0.13^{b}	29.94 ± 0.24^{a}	< 0.001
C18:1n9c	19.77 ± 0.34^{b}	21.18 ± 0.53^{a}	21.58 ± 0.86^{a}	0.010
C18:1n7	2.92 ± 0.03^{a}	2.61 ± 0.04^{b}	2.32 ± 0.03^{c}	< 0.001
C20:1n9	3.05 ± 0.10^{a}	2.74 ± 0.04^{b}	2.35 ± 0.03^{c}	< 0.001
Σ MUFA [2]	31.73 ± 0.32	32.23 ± 0.59	31.67 ± 0.96	0.659
C18:2n6c	6.44 ± 0.21^{c}	9.18 ± 0.28^{b}	11.85 ± 0.09^{a}	< 0.001
C18:3n3	1.55 ± 0.04^{a}	1.33 ± 0.03^{b}	1.08 ± 0.00^{c}	< 0.001
C18:4n3	1.81 ± 0.07^{a}	1.70 ± 0.06^{ab}	1.61 ± 0.02^{b}	0.003
C20:5n3	8.23 ± 0.13^{a}	7.43 ± 0.14^{b}	6.81 ± 0.22^{c}	< 0.001
C22:5n3	1.61 ± 0.02^{a}	1.38 ± 0.03^{b}	1.20 ± 0.04^{b}	0.001
C22:6n3	14.93 ± 0.00^{a}	13.14 ± 0.43^{b}	11.77 ± 0.97^{b}	0.006
Σ PUFA [3]	40.15 ± 0.35	39.11 ± 0.50	38.99 ± 1.19	0.209
Σ n-3 [4]	28.58 ± 0.57^{a}	25.22 ± 0.44^{b}	22.81 ± 1.21^{c}	< 0.001
Σ n-6 [5]	11.05 ± 0.28^{c}	13.36 ± 0.09^{b}	15.71 ± 0.15^{a}	< 0.001
Σ n-3/Σ n-6 [6]	2.59 ± 0.11^{a}	1.89 ± 0.02^{b}	1.45 ± 0.08^{c}	< 0.001
Σ n-6/Σ n-3 [7]	0.39 ± 0.02^{c}	0.53 ± 0.01^{b}	0.69 ± 0.04^{a}	< 0.001
Fatty acids (g/100 g WW)				
C20:5n3	0.11 ± 0.01	0.14 ± 0.06	0.10 ± 0.01	0.212
C22:6n3	0.19 ± 0.02	0.25 ± 0.11	0.16 ± 0.02	0.049
EPA + DHA [8]	0.30 ± 0.03	0.30 ± 0.02	0.26 ± 0.03	0.249

[1] ∑ SFA, sum of saturated fatty acids, includes 12:0, 13:0, 14:0 15:0, 16:0 17:0, 18:0, 20:0, 21:0; [2] ∑ MUFA, sum of monounsaturated fatty acids, includes 16:1n-9, 14:1n-5, 17:1n-7, 18:1n-9, 18:1n7, 22:1n-11; [3] ∑ PUFA, sum of polyunsaturated fatty acids, includes 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-6, 18:2n-4, 18:3n-3, 18:3n-6, 18:4n-1, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 21:5n-3, 22:5n-3, 22:5n-6, 22:6n-3; [4] ∑ n-3, sum of n-3 PUFA, includes 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3; [5] n-6, sum of n-6 PUFA, includes 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:5n-6; [6] Σ n-3/Σ n-6, ratio between the sum of n-3 PUFA and n-6 PUFA;[7] Σ n-6/Σ n-3, ratio between the sum of n-6 PUFA and n-3 PUFA; [8] EPA + DHA = eicosapentaenoic acid (20:5n-3) + docosahexaenoic acid (22:6n-3); Values are presented as mean ± standard error of the mean (n = 4); Values in the same row with a common superscript letter differ significantly ($p < 0.05$).

Table 10. Retention (% Intake) of the European seabass fed the experimental diets.

	CTRL	TM50	TM100	*p*-value
C14:0	31.52 ± 3.33 ^{b}	41.77 ± 3.02 ^{a}	37.18 ± 5.95 ^{ab}	0.025
C16:0	50.46 ± 3.95 ^{b}	68.50 ± 5.77 ^{a}	57.12 ± 8.65 ^{ab}	0.010
C18:0	37.15 ± 3.14 ^{b}	49.10 ± 4.46 ^{a}	41.52 ± 6.86 ^{ab}	0.025
∑ SFA	42.16 ± 3.74 ^{b}	57.31 ± 4.74 ^{a}	48.91 ± 7.51 ^{ab}	0.015
C18:1n9c	39.28 ± 30.42 ^{b}	80.84 ± 7.45 ^{a}	74.40 ± 10.73 ^{ab}	0.028
C18:1n7	39.32 ± 8.24	57.64 ± 5.97	59.50 ± 15.38	0.047
C20:1n9	33.42 ± 7.05	46.85 ± 3.25	47.45 ± 12.18	0.070
∑ MUFA	38.86 ± 15.57 ^{b}	69.86 ± 6.25 ^{a}	66.86 ± 10.56 ^{a}	0.005
C18:2n6c	52.12 ± 14.66	54.03 ± 3.39	40.82 ± 10.40	0.216
C18:3n3	32.56 ± 8.70	49.17 ± 3.30	53.15 ± 16.76	0.060
C18:4n3	34.12 ± 5.24 ^{b}	45.32 ± 2.47 ^{a}	42.43 ± 6.72 ^{ab}	0.032
C20:5n3	30.62 ± 6.23	41.63 ± 2.71	40.87 ± 10.31	0.102
C22:5n3	36.14 ± 9.39	49.95 ± 3.05	52.47 ± 15.48	0.117
C22:6n3	34.50 ± 9.08	47.58 ± 2.22	48.88 ± 14.58	0.134
EPA+DHA	32.69 ± 7.76	44.76 ± 2.44	44.95 ± 12.44	0.122
∑ PUFA	33.92 ± 4.01 ^{b}	44.66 ± 2.73 ^{a}	41.07 ± 6.00 ^{ab}	0.022

[1] ∑ SFA, sum of saturated fatty acids, includes 12:0, 13:0, 14:0 15:0, 16:0 17:0, 18:0, 20:0, 21:0; [2] ∑ MUFA, sum of monounsaturated fatty acids, includes 16:1n-9, 14:1n-5, 17:1n-7, 18:1n-9, 18:1n7, 22:1n-11; [3] ∑ PUFA, sum of polyunsaturated fatty acids, includes 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-6, 18:2n-4, 18:3n-3, 18:3n-6, 18:4n-1, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 21:5n-3, 22:5n-3, 22:5n-6, 22:6n-3; [4] ∑ EPA + DHA = eicosapentaenoic acid (20:5n-3) + docosahexaenoic acid (22:6n-3); Values in the same row with a common superscript letter differ significantly ($p < 0.05$).

Table 11. Nutrient gain (mg/kg ABW /day) of the European seabass fed the experimental diets.

	CTRL	TM50	TM100	*p*-value
C14:0	31.52 ± 3.71	39.05 ± 4.61	38.88 ± 6.36	0.104
C16:0	132.37 ± 12.07 ^{b}	180.20 ± 23.46 ^{a}	183.05 ± 28.15 ^{a}	0.018
C18:0	22.19 ± 2.17 ^{b}	29.52 ± 4.04 ^{ab}	30.59 ± 5.17 ^{a}	0.031
∑ SFA [1]	200.34 ± 20.34 ^{b}	266.11 ± 34.22 ^{ab}	269.35 ± 42.10 ^{a}	0.029
C18:1n9c	108.82 ± 84.65 ^{b}	217.35 ± 30.06 ^{ab}	221.59 ± 31.62 ^{a}	0.029
C18:1n7	21.31 ± 4.68	25.67 ± 3.83	24.39 ± 6.45	0.492
C20:1n9	24.97 ± 5.51	28.90 ± 3.30	26.81 ± 7.02	0.615
∑ MUFA [2]	203.65 ± 82.53 ^{b}	333.24 ± 45.14 ^{a}	332.67 ± 52.94 ^{a}	0.025
C18:2n6c	57.13 ± 15.69 ^{b}	90.01 ± 9.63 ^{ab}	102.30 ± 26.43 ^{a}	0.019
C18:3n3	11.79 ± 3.26	13.41 ± 1.49	12.25 ± 3.91	0.749
C18:4n3	16.19 ± 2.65	19.27 ± 1.92	18.31 ± 3.04	0.277
C20:5n3	45.28 ± 9.67	50.97 ± 5.63	48.57 ± 12.47	0.714
C22:5n3	9.14 ± 2.46	10.03 ± 1.07	9.36 ± 2.80	0.845
C22:6n3	58.80 ± 16.02	64.77 ± 5.93	60.38 ± 18.30	0.835
∑ PUFA [3]	242.47 ± 31.77	298.47 ± 31.71	297.69 ± 45.70	0.099
EPA+DHA [4]	104.07 ± 25.68	115.74 ± 11.54	108.95 ± 30.67	0.793

[1] ∑ SFA, sum of saturated fatty acids, includes 12:0, 13:0, 14:0 15:0, 16:0 17:0, 18:0, 20:0, 21:0; [2] ∑ MUFA, sum of monounsaturated fatty acids, includes 16:1n-9, 14:1n-5, 17:1n-7, 18:1n-9, 18:1n7, 22:1n-11; [3] ∑ PUFA, sum of polyunsaturated fatty acids, includes 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-6, 18:2n-4, 18:3n-3, 18:3n-6, 18:4n-1, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 21:5n-3, 22:5n-3, 22:5n-6, 22:6n-3; [4] ∑ EPA + DHA = eicosapentaenoic acid (20:5n-3) + docosahexaenoic acid (22:6n-3); Values in the same row with a common superscript letter differ significantly ($p < 0.05$).

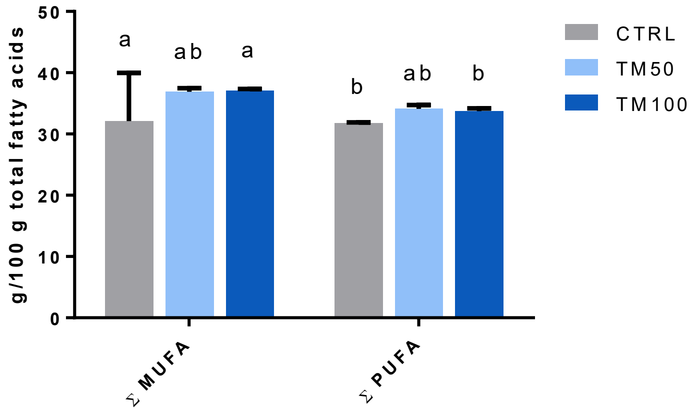

Figure 12. Significant differences in the sum of all the MUFA and PUFA in fish between experimental diets in fish whole-body profile. Bars with a common superscript letter differ significantly ($p < 0.05$). Letters "a" refers to values statistically different and higher than values referred as "b", letters "ab" referred intermediate values. Letters "a" are against "ab" and "b" against "ab" but only within each group, ∑ MUFA and ∑ PUFA.

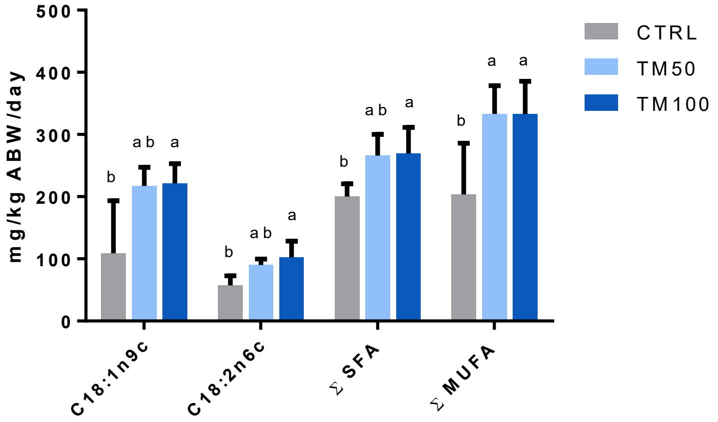

Figure 13. Main differences in nutrient gain (mg/kg ABW /day). Bars with a common superscript letter differ significantly ($p < 0.05$). Letters "a" refers to values statistically different and higher than values referred as "b", letters "ab" referred intermediate values. Letters "a" are against "b" but only within each group, ∑ MUFA and ∑ PUFA.

3. 3. Colour and texture analyses

The tested diets resulted in some chromatic changes in skin and muscle as shown in Table 12. Chroma component, meaning colour saturation, was the only parameter that did not have statistically significant differences between diets. Skin and muscle of fish fed TM50 had the lowest L^* values (40.69 ± 5.32) (*p*= < 0.001). On the contrary, the redness index (a^* values) was highest in skin and muscle of fish fed this diet (TM50; *p*=0.002). The yellowness index (b^* values) were highest in skin and muscle of fish fed TM100 diet (*p*=0.013), and hue values were highest in skin and muscle of fish fed CTRL diet (*p*= < 0.001).

Table 12. Skin and muscle instrumental colour evaluation of European seabass fed experimental diets.

	Experimental diets			
	CTRL	**TM50**	**TM100**	***p*-value**
Skin				
L^*	43.75 ± 3.47^{a}	40.69 ± 5.32^{b}	45.49 ± 6.61^{a}	< 0.001
a^*	-3.95 ± 0.85^{b}	-3.46 ± 0.77^{a}	-3.67 ± 0.77^{ab}	0.002
b^*	3.27 ± 1.37^{b}	3.43 ± 1.11^{b}	4.35 ± 1.55^{a}	0.013
Chroma	5.17 ± 1.48	4.91 ± 1.19	5.75 ± 1.55	0.050
Hue	141.63 ± 6.65^{a}	135.86 ± 7.71^{b}	131.21 ± 7.62^{b}	< 0.001
Muscle				
L^*	40.75 ± 1.83^{a}	39.73 ± 3.18^{b}	40.22 ± 2.10^{a}	< 0.001
a^*	-3.28 ± 0.34^{b}	-2.93 ± 0.62^{a}	-3.08 ± 0.36^{ab}	0.002
b^*	2.17 ± 0.48^{b}	2.23 ± 0.65^{b}	2.47 ± 0.92^{a}	0.013
Chroma	3.96 ± 0.43	3.78 ± 0.48	4.04 ± 0.57	0.050
Hue	146.62 ± 5.58^{a}	142.63 ± 12.17^{b}	142.19 ± 11.03^{b}	< 0.001

Values are presented as mean ± standard error of the mean (n = 4); Values in the same row with a common superscript letter differ significantly (*p* < 0.05).

The results of instrumental textural measurements (hardness, adhesiveness, springiness, cohesiveness, chewiness, and resilience) are presented in Table 13. Only chewiness (N) showed significant differences among dietary treatments (*p*=0.049), fish fed TM100 (0.38 + 0.08 N) had higher values than those fed TM50 (0.59 ± 0.28 N) but did not differ significantly from CTRL (0.47 + 0.15 N).

Table 13. Texture profile parameters analysis of European seabass fed experimental diets.

	Experimental diets			
	CTRL	**TM50**	**TM100**	***p*-value**
Hardness (N)	0.90 ± 0.12	0.92 ± 0.19	0.83 ± 0.10	0.189
Adhesiveness (J)	-0.03 ± 0.02	-0.02 ± 0.02	-0.03 ± 0.02	0.460
Springiness	1.28 ± 0.27	1.35 ± 0.39	1.11 ± 0.13	0.088
Cohesiveness	0.39 ± 0.05	0.44 ± 0.07	0.41 ± 0.04	0.538
Chewiness (J)	0.47 ± 0.15^{ab}	0.59 ± 0.28^{b}	0.38 ± 0.08^{a}	0.043
Resilience	0.40 ± 0.09	0.38 ± 0.12	0.38 ± 0.13	0.506

Values are presented as mean ± standard error of the mean (n = 4); Values in the same row with a common superscript letter differ significantly ($p < 0.05$).

3. 4. Sensory analysis

There were no significant differences in the overall acceptance between the samples evaluated by the sensory panel. The position of the batch did not have a significant effect on the acceptance of samples by the tasters. Overall, consumers liked the product (Table 14; more than 90% positive comments). All the samples were positively characterized by their pleasant aspect, texture, and flavour. The CTRL sample seems to be associated with a whiter and pleasant colour. The TM50 and TM100 samples were associated with a juicy texture (Table 14). Relatively to negative characteristics, there were no associations with statistical relevance. Also, there were no differences in the global acceptance level between seabass fed with different types of diets (Figure 14). All seabass tested had positive ratings and were accepted by most tasters. It was not detected an impact of fish slice in the global acceptance of the samples.

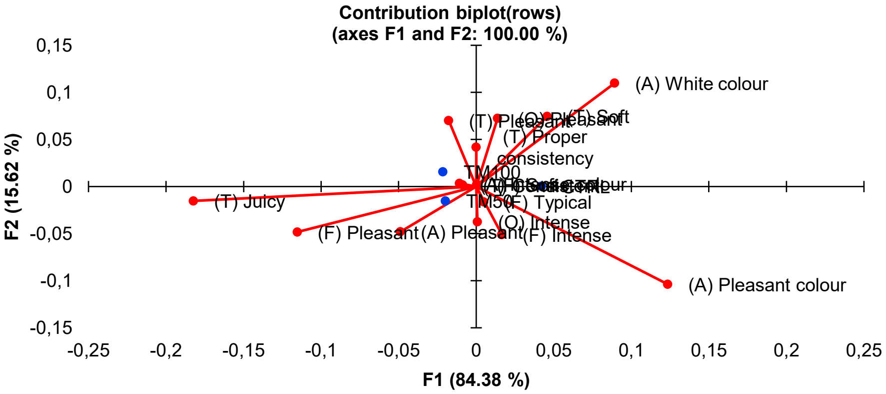

Figure 14. Correspondence analysis biplot of the mean scores and loadings of European seabass fed the experimental diets (CTRL, TM50, TM100) applied to assess the consensus among the variables that differed significantly among dietary treatments in terms of consumer acceptance of seabass fillets.

Table 14. Analysis of the consumer's comments.

	Experimental diets			
	CTRL	**TM50**	**TM100**	**Total**
Positive	171	180	170	521
Negative	12	6	9	27
Positive		***Appearance***		
Pleasant	21	24	22	67
Pleasant colour	13	9	6	28
White colour	12	7	9	28
Intense colour	2	3	3	8
		Odour		
Pleasant	20	20	20	60
Intense	2	3	1	6
		Texture		
Soft	21	20	20	61
Pleasant	19	20	20	59
Juicy	8	16	15	39
Proper consistency	2	1	3	6
Consistent	1	2	2	5
		Flavour		
Pleasant	41	46	43	130
Intense	5	5	3	13
Soft	2	2	2	6
Typical	2	2	1	5
Negative				
Too dry	4	1	3	8
Too soft	1	-	4	5
Little intense	4	1	3	8
Not characteristic	1	4	1	6

4. Discussion

The global demand for animal protein over the past few years launched an effort to increase the development in the feedstuff industry to keep up with aquaculture intensive production needs, especially for carnivorous fish species as *Dicentrarchus labrax* (Grigorakis, 2007; Kousoulaki et al., 2015). Despite the reductions in the amounts of FM used in today's diets, this effort must continue in order to privilege more sustainable formulations that cover the species without harming the welfare and nutritional quality of the fish (Iaconisi et al., 2018; Nogales-Mérida et al., 2019).

Among all terrestrial ingredients able to replace FM, such as plant proteins, insects have been an up and coming ingredient not only for their ecological footprint in terms of production but also for all their nutritional quality regarding protein and lipid levels (Henry et al., 2015). To ensure the feasibility of insects as a substitute for FM it is important to assess the quality-defining parameters as to how it interferes with the growth, development, and nutritional quality of fish fed with experimental diets and to appraise the technical quality with consumer's perception when these types of substitutions are made. Nutritional quality is mainly related to fillets PUFA content and sensorial quality more approached to organoleptic and sensory perception of consumers (Grigorakis, 2017; Piccolo et al., 2017). Few studies have evaluated the total replacement of FM by insect meal in diets for marine fish species and to the best of our knowledge, the present study was the first one to address the effects of total replacement of FM by defatted *T. molitor* larvae meal in European seabass diets.

Remarking all the results obtained in the present study, the major advances are related to the generally good growth performance and somatic indexes observed in all fish fed the experimental diet. All fish had similar final weight (around 267 g) and our results generally agree with Chemello et al. (2020) that demonstrated that the total replacement of FM by *T. molitor* did not affect growth performance of rainbow trout. Iaconisi et al. (2017b) also reported no overall effects in growth performance of blackspot sea bream (*Pagellus bogaraveo*) fed with diets that had 25% and 50% of *T. molitor* inclusion. Likewise, Piccolo et al. (2017b) did not report any negative effects on growth performance of gilthead seabream (*Sparus aurata)* fed diets with 25% and 50% *T. molitor* to replace FM. On the other hand, Ido et al. (2019) carried out a study with 100% replacement of FM by *T. molitor* in red seabream (*Pargus major*) achieving positive results in overall growth performance and disease resistance, justifying the improvement with the defatting process of diets used in the experiment. In contrast to all these studies, Gasco et al. (2016) reported an impairment of growth performance parameters in European seabass with the dietary inclusion of 50% of a full-fat *T. molitor* meal, decreasing DM intake, feeding rate, and weight gain. Regarding the use of other insect species, Abdel-tawwab et al. (2020) tested dried *Hermetia illucens* in European seabass diets (12.1 ± 0.21 g) and reported no significant differences on growth performance with protein replacement of 25, 35, or 50% of FM. These authors have also performed a simple economic analysis of fish production and reported that diets with increasing levels of black soldier fly (BSF)decreased their feeding cost by 15.6% per ton of fish gain, implying a decrease from 0.71 to 0.60 US$ (EUR 0.60 to 0.51) per kg diet. They explained this reduction by the prices of feed, feeding rate, stocking density, fish size, fish yield, and fish sales.

Regarding VFI, our results evidenced a decrease in this parameter with insects' inclusion, especially in TM50. Belforti et al. (2015) also reported a VFI decrease in rainbow trout fed with diets with full-fat *T. molitor*. This reduction was associated with the high-fat content present in insect-based diets and/or the type of fatty acids in those diets. However, this was not the case in the present study as diets were isolipidic. It can also be hypothesized that increased dietary lipid content might have contributed to fatty acid oxidation that might have led to a reduced appetite, and consequently, reduced feed intake (Belghit et al., 2019). Rema et al. (2019) have also observed reduced feed intake but, like in the present study, diets were isolipidic, so they suggested that this was probably correlated with a better nutritional and metabolic adequacy of such diets since this reduction on intake did not decrease growth performance and even increased feed efficiency.

In the present study, the substitution of FM by increasing levels of TM significantly affected the FCR and PER, which give insect diets a great advantage from a mass production perspective, as the TM50 diet showed the lowest FCR and the TM100 diet the same FCR as the control, thus giving a possibility for future replacement of FM. Also, the fact that seabass in TM50 treatment had a lower FCR than those fed the CTRL diet, implies that fish had lower feed wastage and/or higher utilization of feed. Likewise, Belforti et al. (2015) showed a low FCR and a higher PER with 25% and 50% of full-fat *T. molitor* inclusion in diets for rainbow trout. Contrarily, Mikołajczak et al. (2020) reported decreased PER in sea trout (*Salmo trutta* m. *trutta*) fingerlings fed with diets containing 10% of hydrolysed mealworm. In juvenile rainbow trout, Rema et al. (2019) have evaluated the total replacement of FM by *T. molitor* and have also observed a lower FCR and an increased PER in diets with TM when compared to a control diet, confirming the present results. The PER increment may be related to increased protein digestibility. This is particularly interesting since insects' crude protein digestibility is normally lower than FM, probably due to chitin content present in insects (estimated to 6.6% of total nitrogen in mealworm larvae meals) (Ido et al., 2019), but that was not evaluated in our study. Basto et al. (2020) have recently determined the ADC of commercially available insect larvae species (BSF, TM and locust meal (LM)), full-fat and defatted. Results highlighted the high protein level, high protein digestibility and high amount of digestible EAAs in *Tenebrio molitor* larvae meals (> 89%) suggesting they were promising protein sources for European seabass, preferentially in their defatted form.

Regarding European seabass whole-body proximate composition, crude fat increased after insects' inclusion. Although insects have a high-fat content, that in the case of *T. molitor* can reach 30-35 % DM (Piccolo et al. 2017), the TM used in the present trial was defatted and diets remained isolipidic, so the increased fat content was probably due to differences in metabolism. Ng (2001) also reported higher lipid content in African catfish (*Clarias gariepinus*) fed diets containing *T. molitor* to replace 20, 40, 60, 80 and 100% of FM compared with the control.

It is well established that the dietary fatty acid profile will be reflected in the fatty acid profile of fish tissues (Bruni et al., 2020; Kousoulaki et al., 2015; Renna et al., 2017). It is also important to refer that differences in fatty acid profiles between studies, even when using the same insect species, are normal to occur since many factors can affect insects composition (Borgogno et al., 2017; Iaconisi et al., 2018; Renna et al., 2017). For example, the rearing substrates used for growing insects are very important since insects can accumulate different fatty acids, and therefore their fatty acid profile can be tailored according to the requirements needed to be used in fish trials (Paul et al., 2017). Ewald et al. (2020) used different diets containing marine products for producing BSL larvae and concluded that their fatty acid profile can be affected by diets, but these modifications are limited since the EPA and DHA percentage incorporated in the larval stage decreased with weight gain. These findings suggest that fish oil substitutions can be compromised with the inclusion of insect meals, but they can still be suitable for vegetable oils substitutions.

Generally, fish have low SFA content (<30%), except for some fatty species (Alasalvar et al., 2002). The present study showed that muscle SFA had significantly higher values when the TM100 diet was used, indicating that insect inclusion may induce the increase of SFA, confirming previous data reported in the literature (Borgogno et al., 2017). Palmitic acid (C16:0) had the highest percentage among the SFA and significantly increased with *T. molitor* inclusion. One positive side for this increment is the fact that fish are different from other vertebrates since the energy is predominantly obtained from C16:0 and MUFA (C18:1n9, C20:1n11, C22:1n11), so TM50 and TM100 diets had more energy available for muscle deposition and growth (Bruni et al., 2020; Tocher, 2003). Contrarily to the present observations where TM100 samples presented significantly higher values for these fatty acids when compared with TM50 and CTRL, Gasco et al. (2016) and Iaconisi et al. (2018) reported that the sum of SFA in European seabass and rainbow trout muscle was not influenced by the dietary inclusions of *T. molitor* (25 and 50% of inclusion levels in both trials) even when the ingredient and diets formulation included high SFA content.

Oleic acid (C18:1n9c) is the most abundant fatty acid among dietary MUFA, and high inclusions of insects increased their percentage in muscle lipids. This happened possibly because this fatty acid is present in high content in insects, especially in *Tenebrio molitor* (Iaconisi et al., 2018; Nogales-Mérida et al., 2019). Despite these differences, the sum of MUFA did not have significant differences among dietary treatments since other MUFA decreased with increasing levels of *T. molitor.*

In seabass, the linoleic acid (18:2n6) has the highest percentage among all the PUFA, with TM100 presenting the highest values, reflecting the dietary profile. This happens in other studies and is explained by the high dietary inclusion of terrestrial ingredients in aquafeeds (Belforti et al., 2015; Grigorakis, 2007; Iaconisi et al., 2018, 2017b; Nogales-Mérida et al., 2019; Skalli et al., 2006). Another possible explanation for these high amounts of linoleic acid is due to the lack of desaturase and elongase activity that would improve the biosynthesis of LC-PUFA from linoleic acid (Glencross, 2009). Also, it is important to highlight that dietary linoleic acid may lead to oxidation of LDL and increased production of pro-inflammatory mediators via metabolic conversion to arachidonic acid (ARA, C20:4n-6) (Simopoulos, 2008).

The n-3 percentage decreased in the muscle of fish fed TM, resulting in reduced n-3/n-6 ratio, and this is in agreement with previous data (Alasalvar et al., 2002; Barroso et al., 2014; Belforti et al., 2015; Iaconisi et al., 2018). Khosravi et al. (2018) have also reported decreased n-3 PUFA in fillets of rockfish (*Sebastes schlegeli*) fed with increasing levels of *T. molitor* (8, 16, 24, and 32%). According to Gasco et al. (2019), fish fed with high levels of TM meal have shown a decrease in the n-6 PUFA content, which did not happen with our results, and is probably associated with distinct dietary formulations among studies.

European seabass, like other marine fish species, cannot elongate and desaturate C18 fatty acids to EPA and DHA, due to the lack of 12 and 15 desaturases. Thus, they cannot synthetize these fatty acids *de novo*, and their right amounts should be included in the diets (Kousoulaki et al., 2015; Tocher, 2003; Turchini et al., 2013). All experimental diets have fish oil in their formulation (high amounts in CTRL and decreasing in insect diets), but the percentage of the essential fatty acids EPA and DHA decreased with insect inclusion in experimental diets. The amount of these two fatty acids even in TM50 and TM100 diets are related to the fish oil content, due to the lack of EPA and DHA in insects, the biggest limitation in insects usage in diets for marine fish (Barroso et al., 2014). Previous studies have also reported a decrease in EPA+DHA content in the muscle when replacing fishmeal with insects as a protein source. Belforti et al. (2015) reported this decrease with inclusion levels of 25 and 50 % of *T. molitor* in rainbow trout diets. Nevertheless, the total amount of EPA and DHA in European seabass whole-body and 100g portion of fillet did not have significant differences when expressed by g/100 g of WW. This happens because, although the relative % of EPA and DHA in terms of total fatty acids decreased, the fillet lipid content increased both in whole-body and muscle, contributing to increased total amounts of these fatty acids in the fillet. In our study, the EPA+DHA values for muscle in fish fed the tested diets complied with the EFSA recommendation for a daily intake of 0.25 g/100 g fresh weight of EPA+DHA for healthy individuals in both TM50 and TM100 diets (EFSA, 2012).

According to Tocher, (2015), oxidation of fatty acids is related to enzyme specificities and competition between fatty acids, meaning that for the proper retention of preferential fatty acid, the right balance needs to be established. Fatty acids retention may fluctuate taking into account several factors as fish species, nutritional status, or the dietary composition. Different fatty acids may have different retention levels, and it is known that, for example, EPA and DHA do not change a lot their retention levels, regardless of the lipid source used in the diets. On the other hand, linoleic acid (C18:2n6) and linolenic acid (C18:n3) have selective retention for different lipid sources and has been demonstrated to be preferentially retained (Glencross, 2009). Asuman et al. (2016) reported in a study of different feeding schedules for European seabass that since the linoleic acid is deposited in the whole body, it is also selectively retained in fish flesh and is resistant to suffer desaturation, even if the dietary lipid source is changed. This was observed in our study since the consumption and retention levels were significantly higher for some fatty acids, as oleic and linoleic acid that was present in muscle and whole body in high amounts. Fish fed TM50 had higher retention of oleic and linoleic acid, probably due to a lower feed intake, but resulted in the highest gain in fish fed TM100.

Fish colour may vary according to several factors including diet, temperature or season, and type of storage (Fuentes et al., 2010; Iaconisi et al., 2018). Diets used in this trial seem to have an impact on skin and fillet colour. Skin and muscle *L** values for fish fed TM50 diet were lower than TM100 and CTRL. Fuentes et al. (2010) compared skin colour of wild and farmed seabass reporting that wild seabass (42.39 ± 1.54) was lighter than farmed seabass (37.63 ± 4.69; 36.66 ± 3.62). The muscle *L** values presently observed are similar to those observed in wild seabass. Regarding *a** values, it is important to highlight the presence of β-carotene pigment in insects that is expressed as a red-coloured pigment. Since fish are not able to produce carotenoids *de novo*, those who are present in skin and muscle are exclusively inherent to experimental diets (Iaconisi et al., 2017). Although seabass has white flesh, it is important to notice that *b** values increased between diets, which means that both skin and muscle turned yellowish, being significantly higher in the TM100 diet. One possible explanation for this effect may be the higher muscle lipid content in those fish. Also, this may be due to the presence of riboflavin (vitamin B2) in mealworms, a yellow-coloured pigment, that when used in high inclusion levels may lead to increased deposition in fish fillets and skin. Increased *b** values were also observed by Iaconisi et al. (2017b), with the inclusion of 25% and 50% of *T. molitor* in blackspot sea bream diets. Despite all this, both diets (TM50 and TM100) correspond to colour values reported by Fuentes et al. (2010) for wild seabass skin. In summary, the present results are in general accord with Iaconisi et al. (2017) results, reporting increased redness in the fillet of blackspot seabream fed with the 33% dietary inclusions of *T. molitor* replacing 25% of FM.

The chemical parameters and sensory evaluation are strongly linked. Fatty acid and lipid profile have been reported to affect texture attributes like texture, juiciness, and tenderness (Borgogno et al., 2017). According to Arechavala-Lopez et al. (2013) texture parameters as cohesiveness, springiness and hardness are normally higher in wild seabass than in farmed fish. In our study, these parameters did not have significant differences between dietary treatments. However, chewiness increased with the inclusion of *T. molitor*, indicating an improvement of fillet quality with insect-based diets. Likewise, Mancini et al. (2018) and Bruni et al. (2020) also did not report overall differences in the texture of fish flesh (rainbow trout and Atlantic salmon, respectively) fed with black soldier fly (25 and 50% of substitution of FM and 4.91, 9.84, and 14.75% of BSF inclusion, respectively). Overall, in this study, TM larvae meal did not influence the texture characteristics of fillets. This is in general accord with previous studies where 25 and 50% of FM were substituted by TM in diets for blackspot seabream (Iaconisi et al., 2017), gilthead seabream (Piccolo et al., 2017), and rainbow trout (Iaconisi et al., 2018).

Fish muscle represents the edible part of the fish (44.2 % of seabass whole-body) and its lipid content plays an important role in sensory profile since it is responsible for a certain flavour of fish flesh, due to its volatile compounds characteristic of fish flavour (Arechavala-Lopez et al., 2013; Gasco et al., 2019; Grigorakis, 2007). According to Grigorakis (2007), fatty fish are normally associated with a “juicy” sensation, meaning that a high lipid content may affect the fish taste. In the present study, and according to the consumer’s comments, fish fed with TM50 and TM100 had the highest juiciness when compared with CTRL. In a study performed by Mancuso et al. (2016), where the Italian consumers' interest in fish fed with insects was analysed, 90% of the inquiries responded that they intended to purchase those products if the hygiene and quality requirements were fulfilled. In our study, the terms freely provided by the panellists to describe fish sensory properties were not related to any negative hedonic valence, which may indicate that a partial or full FM replacement with insect meal did not lead to a perception of sensory defects or off-flavours. These results are especially important from a market perspective, since consumers, will be able to buy a fish that was fed with insects without perceiving any differences in flavours. This may be related to the defatting process of the insect meal that has been suggested as a method to improve insects’ organoleptic characteristics (Mastoraki et al., 2020; Sánchez-Muros, Barroso, and Haro, 2016). Arechavala-Lopez et al. (2013) affirm that farmed seabass seems to have higher juiciness than wild ones, which may also explain the comments of the panellists. Stadtlander et al. (2017) also performed a sensory evaluation of rainbow trout fed with different levels of black soldier fly but no significant differences were found by panellist for taste and odour. These are very promising results as the observed differences in muscle fatty acids do not seem to be perceived by consumers.

5. Conclusion

The present study demonstrated that the substitution of 50% FM by TM significantly improves FCR and PER, without affecting fish growth. Likewise, total replacement of FM by TMd did not impair European seabass growth performance, HSI and VSI, but increased the whole-body fat content, suggesting a strong impact on lipid metabolism. On the other hand, it is important to highlight that fish fed insect-based diets had a positive impact on consumers' view since no differences were found in all the parameters evaluated in the sensory perception of the fillet.

Despite the reduction of the relative content of EPA and DHA (% total fatty acids) in the muscle, the absolute values of EPA + DHA in a fillet portion of 100 g for human consumption remained above the recommended levels for human consumption (>0.25g / 100g of wet weight) in all fish and did not vary significantly among treatments. These results suggest a possible replacement of fish meal with *Tenebrio molitor* in diets for European seabass, but the total substitution of FM has to be addressed with caution in a long-term trial.

6. Future perspectives

Some studies are needed to fully understand the utilization of fatty acids and other nutrients present in insect meals and the way they influence seabass general condition. For example, it will be important to evaluate nutrient digestibility to better understand ingredient utilization by fish and ascertain if chitin might have some influence on intestinal structure or function (e.g. any probiotic effect).

The evaluation of enzyme activities would be another important study to conduct, especially when related to lipid metabolism, as well as, measuring the lipid content of liver to better comprehend fish lipogenesis and lipid deposition and metabolism.

7. References

Abbate, F., M.C. Guerrera, G. Montalbano, F. De Carlos, A.Á. Suárez, E. Ciriaco, A. Germanà. 2012. 'Morphology of the European sea bass (*Dicentrarchus labrax*) tongue'. *Microscopy Research and Technique* 75(5): 643–649. https://doi.org/10.1002/jemt.21105.

Abdel-Tawwab, M., R.H. Khalil, A.A. Metwally, M.S. Shakweer, M.A. Khallaf, H.M.R. Abdel-latif. 2020. 'Effects of black soldier fly (*Hermetia illucens* L.) larvae meal on growth performance, organs-somatic indices, body composition, and hemato-biochemical variables of European Sea Bass, *Dicentrarchus labrax*'. *Aquaculture* 522: 735136. https://doi.org/10.1016/j.aquaculture.2020.735136.

Ait-Yahia, D., S. Madani, J.L. Savelli, J. Prost, M. Bouchenak, J. Belleville. 2003. 'Dietary fish protein lowers blood pressure and alters tissue polyunsaturated fatty acid composition in spontaneously hypertensive rats'. *Nutrition* 19(4): 342–346. https://doi.org/10.1016/S0899-9007(02)00858-4

Al-Qazzaz, M.F., D.B. Ismail. 2016. 'Insect meal as a source of protein in animal diet'. *Animal Nutrition and Feed Technology* 16(3): 527–547. doi: 10.5958/0974-181X.2016.00038.X

Alasalvar, C., K.D.A. Taylor, E. Zubcov, F. Shahidi, M. Alexis. 2002. 'Differentiation of cultured and wild Sea Bass (*Dicentrarchus labrax*): Total lipid content , fatty acid and trace mineral composition'. *Food Chemistry*, 79: 145–150. https://doi.org/10.1016/S0308-8146(02)00122-X

Alvergne, A., C. Jenkinson, C. Faurie. 2016. 'Evolutionary thinking in medicine'. *Evolutionary Thinking in Medicine*.

Arechavala-Lopez, P., D. Fernandez-Jover, K.D. Black, E. Ladoukakis, J.T. Bayle-Sempere, P. Sanchez-Jerez, T. Dempster. 2013. 'Differentiating the wild or farmed origin of Mediterranean fish: a review of tools for Sea Bream and Sea Bass'. *Reviews in Aquaculture* 5(3): 137–157. https://doi.org/10.1111/raq.12006

Arru, B., R. Furesi, L. Gasco, F.A. Madau, P. Pulina. 2019. 'The introduction of insect meal into fish diet: the first economic analysis on European Sea Bass farming'. *Sustainability (Switzerland)* 11(6): 1–16. https://doi.org/10.3390/su11061697

Asuman, H., G. Corraze, S. Panserat, O. Tufan. 2016. 'Effects of alternate feeding with different lipid sources on fatty acid composition and bioconversion in European Sea Bass (*Dicentrarchus labrax*)'. *Aquaculture* 464: 28–36. https://doi.org/10.1016/j.aquaculture.2016.06.013

Ayala, M.D., O. López Albors, A. Blanco, A. García Alcázar, E. Abellán, G. Ramírez Zarzosa, F. Gil. 2005. 'Structural and ultrastructural changes on muscle tissue of Sea Bass, *Dicentrarchus labrax L.*, after cooking and freezing'. *Aquaculture* 250(1–2): 215–231. https://doi.org/10.1016/j.aquaculture.2005.04.057

Barroso, F.G., C. de Haro, M.J. Sánchez-Muros, E. Venegas, A. Martínez-Sánchez, C. Pérez-Bañón. 2014. 'The potential of various insect species for use as food for fish'. *Aquaculture* 422–423: 193–201. http://dx.doi.org/10.1016/j.aquaculture.2013.12.024.

Basto, A., E. Matos, L.M.P. Valente. 2020. 'Nutritional value of different insect larvae meals as protein sources for European Sea Bass (*Dicentrarchus labrax*) juveniles'. *Aquaculture* 521: 735085. https://linkinghub.elsevier.com/retrieve/pii/S0044848619334532.

Belforti, M., F. Gai, C. Lussiana, M. Renna, V. Malfatto, L. Rotolo, M. De Marco, S. Dabbou, A. Schiavone, I. Zoccarato, L. Gasco. 2015. '*Tenebrio molitor* meal in Rainbow Trout (*Oncorhynchus mykiss*) diets: Effects on animal performance, nutrient digestibility and chemical composition of fillets'. *Italian Journal of Animal Science* 14(4): 670–676. https://doi.org/10.4081/ijas.2015.4170

Belghit, I., N.S. Liland, P. Gjesdal, I. Biancarosa, E. Menchetti, Y. Li, R. Waagbø, Å. Krogdahl, & E.J. Lock. 2019. 'Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic Salmon (*Salmo salar*)'. *Aquaculture* 503: 609–619. https://doi.org/10.1016/j.aquaculture.2018.12.032.

Belghit, I., N.S. Liland, R. Waagbø, I. Biancarosa, N. Pelusio, Y. Li, Å. Krogdahl, E.J. Lock. 2018. 'Potential of insect-based diets for Atlantic Salmon (*Salmo salar*)'. *Aquaculture* 491: 72–81. https://doi.org/10.1016/j.aquaculture.2018.03.016.

Belghit, I., R. Waagbø, E.J. Lock, N.S. Liland. 2019. 'Insect-based diets high in lauric acid reduce liver lipids in freshwater Atlantic Salmon'. *Aquaculture Nutrition* 25(2): 343–357. https://doi.org/10.1111/anu.12860

Borgogno, M., C. Dinnella, V. Iaconisi, R. Fusi, C. Scarpaleggia, A. Schiavone, E. Monteleone, L. Gasco, G. Parisi. 2017. 'Inclusion of *Hermetia illucens* larvae meal on Rainbow Trout (*Oncorhynchus mykiss*) feed : Effect on sensory profile according to static and dynamic evaluations'. *Journal of the Science of Food and Agriculture* 97(10): 3402–3411. https://doi.org/10.1002/jsfa.8191

Bosch, G., H.H.E. Van Zanten, A. Zamprogna, M. Veenenbos, N.P. Meijer, H.J. Van Der Fels-klerx, J.J.A. Van Loon. 2019. 'Conversion of organic resources by black soldier fly larvae: Legislation, Efficiency and Environmental Impact'. *Journal of Cleaner Production* 222: 355–363. https://doi.org/10.1016/j.jclepro.2019.02.270.

Boukortt, F.O., A. Girard, J. Prost, D. Ait-Yahia, M. Bouchenak, J. Belleville. 2004. 'Fish protein improves the total antioxidant status of Streptozotocin - Induced diabetes in spontaneously hypertensive rat'. *Medical Science Monitor* 10(11): 397–405. BR397-BR404.

Bruni, L., I. Belghit, E.J. Lock, G. Secci, C. Taiti, G. Parisi. 2020. 'Total replacement of dietary fish meal with black soldier fly (*Hermetia illucens*) larvae does not impair physical, chemical or volatile composition of farmed Atlantic Salmon (*Salmo salar L.*)'. *Journal of the Science of Food and Agriculture* 100(3): 1038–1047. https://doi.org/10.1002/jsfa.10108

Bruni, L., B. Randazzo, G. Cardinaletti, M. Zarantoniello, F. Mina, G. Secci, F. Tulli, I. Olivotto, G. Parisi. 2020. 'Dietary inclusion of full-fat *Hermetia illucens* prepupae meal in practical diets for Rainbow Trout (*Oncorhynchus mykiss*): Lipid metabolism and fillet quality investigations'. *Aquaculture* 529: 735678. https://doi.org/10.1016/j.aquaculture.2020.735678.

Bugeon, J., F. Lefevre, M. Cardinal, A. Uyanik, A. Davenel, P. Haffray. 2010. 'Flesh quality in large rainbow trout with high or low fillet yield'. *Journal of Muscle Foods* 21(4): 702–721 https://doi.org/10.1111/j.1745-4573.2010.00214.x.

Burlingame, B., C. Nishida, R. Uauy, R. Weisell. 2009. 55 Annals of Nutrition and Metabolism *Fats and Fatty Acids in Human Nutrition: Introduction*. https://doi.org/10.1159/000228993

Cairone, F., S. Carradori, M. Locatelli, M.A. Casadei, S. Cesa. 2020. 'Reflectance Colorimetry: a mirror for food quality—a Mini Review'. *European Food Research and Technology* 246(2): 259–272. https://doi.org/10.1007/s00217-019-03345-6.

Campos, I., E. Matos, A. Marques, L.M.P. Valente. 2017. 'Hydrolyzed feather meal as a partial fishmeal replacement in diets for European Seabass (*Dicentrarchus labrax*) juveniles'. *Aquaculture* 476: 152–159. http://dx.doi.org/10.1016/j.aquaculture.2017.04.024.

Campos, I., E. Matos, L.M.P. Valente. 2019. ‘Ability of European Seabass (*Dicentrarchus labrax*) to digest rendered animal fats from fish, poultry and mammals’. *Aquaculture Nutrition* 25(3): 729–736. https://doi.org/10.1111/anu.12902

Cardinaletti, G., B. Randazzo, M. Messina, M. Zarantoniello, E. Giorgini, A. Zimbelli, L. Bruni, G. Parisi, I. Olivotto, F. Tulli. 2019. ‘Effects of graded dietary inclusion level of full-fat *Hermetia illucens* prepupae meal in practical diets for Rainbow Trout (*Oncorhynchus mykiss*)’. *Animals* 9(5): 1–21. https://doi.org/10.3390/ani9050251

Cashion, T., F. Le Manach, D. Zeller, D. Pauly. 2017. ‘Most fish destined for fishmeal production are food-grade fish’. *Fish and Fisheries* 18(5): 837–844. https://doi.org/10.1111/faf.12209

Chemello, G., M. Renna, C. Caimi, A. Oliva-teles. 2020. ‘Partially defatted *Tenebrio molitor* larva meal in diets for grow-out Rainbow Trout, *Oncorhynchus mykiss* (Walbaum): effects on growth performance, diet digestibility and metabolic responses’. *Animals*. https://doi.org/10.3390/ani10020229

Cheng, J.H., D.W. Sun, Z. Han, X.A. Zeng. 2014. ‘Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: A Review’. *Comprehensive Reviews in Food Science and Food Safety* 13(1): 52–61. https://doi.org/10.1111/1541-4337.12043

Chéret, R., N. Chapleau, C. Delbarre-Ladrat, V. Verrez-Bagnis, M. de Lamballerie. 2005. ‘Effects of high pressure on texture and microstructure of Sea Bass (*Dicentrarchus labrax* L.) fillets’. *Journal of Food Science* 70(8). https://doi.org/10.1111/j.1365-2621.2005.tb11518.x

Choi, B.D., N.A.K. Wong, & J.-H. Auh. 2017. ‘Defatting and sonication enhances protein extraction from edible insects.’ *Korean Journal for Food Science of Animal Resources* 37(6): 955–961. doi: 10.5851/kosfa.2017.37.6.955

Claret, A., L. Guerrero, I. Gartzia, M. Garcia-Quiroga, R. Ginés. 2016. ‘Does information affect consumer liking of farmed and wild fish?’ *Aquaculture* 454: 157–162. http://dx.doi.org/10.1016/j.aquaculture.2015.12.024.

Clavelle, T., S.E. Lester, R. Gentry, H.E. Froehlich. 2019. ‘Interactions and management for the future of marine aquaculture and capture fisheries’. *Fish and Fisheries* (December 2018): 368–388. https://doi.org/10.1111/faf.12351

Clydesdale, F.M. 1991. ‘Color perception and food quality’. *Journal of Food Quality* 14(1): 61–74. https://doi.org/10.1111/j.1745-4557.1991.tb00047.x

Coppes, Z., A. Pavlisko, S. De. 2002. ‘Journal of aquatic food texture measurements in fish and fish products’. *Journal of Aquatic Food Product Technology* 11(1): 89–105. https://doi.org/10.1300/J030v11n01_08

Costa, S., S. Pedro, H. Lourenço, I. Batista, B. Teixeira, N.M. Bandarra, D. Murta, R. Nunes, C. Pires. 2020. ‘Evaluation of *Tenebrio molitor* larvae as an alternative food source’. *NFS Journal* 21: 57–64. https://doi.org/10.1016/j.nfs.2020.10.001

Coutteau, P., G. Van Stappen, P. Sorgeloos. 1996. ‘A standard experimental diet for the study of fatty acid requirements of weaning and first ongrowing stages of the European Sea Bass *Dicentrarchus labrax L.*: Comparison of extruded and extruded/coated diets’. *Archives of Animal Nutrition* 49(1): 49–59. https://doi.org/10.1080/17450399609381863

Dias, J., M.J. Alvarez, J. Arzel, G. Corraze, A. Diez, J.M. Bautista, S.J. Kaushik. 2005. ‘Dietary protein source affects lipid metabolism in the European Seabass (*Dicentrarchus labrax*)’. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 142(1): 19–31. https://doi.org/10.1016/j.cbpb.2005.07.005

Dias, J., M.J. Alvarez, A. Diez, J. Arzel, G. Corraze, J.M. Bautista, S.J. Kaushik. 1998. ‘Regulation of hepatic lipogenesis by dietary protein/energy in juvenile European Seabass (*Dicentrarchus labrax*)’. *Aquaculture* 161(1–4): 169–186. https://doi.org/10.1016/S0044-8486(97)00268-8

Dumas, A., T. Raggi, J. Barkhouse, E. Lewis, E. Weltzien. 2018. ‘The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of Rainbow Trout (*Oncorhynchus mykiss*)’. *Aquaculture* 492: 24–34. https://doi.org/10.1016/j.aquaculture.2018.03.038.

EFSA. 2012. 'Scientific opinion on the tolerable upper level of eicosapentanoic acid (EPA), docosahexaenoic acis (DHA) and docosapentaenoic acids (DPA)'. *EFSA Journal* 10(7): 1-48. doi: 10.2903/j.efsa.2012.2815

Elvevoll, E.O., H. Barstad, E.S. Breimo, J. Brox, K.E. Eilertsen, T. Lund, J.O. Olsen, B. Østerud. 2006. ‘Enhanced incorporation of N-3 Fatty acids from fish compared with fish oils’. *Lipids* 41(12): 1109–1114. https://doi.org/10.1007/s11745-006-5060-3

Enes, P., S. Panserat, S. Kaushik, A. Oliva-Teles. 2011. ‘Dietary Carbohydrate Utilization by European Sea Bass (*Dicentrarchus labrax L.*) and Gilthead Sea Bream (*Sparus aurata* L.) juveniles’. *Reviews in Fisheries Science* 19(3): 201–215. https://doi.org/10.1080/10641262.2011.579363

Eroldoğan, O.T., M. Kumlu, & M. Aktaş. 2004. ‘Optimum feeding rates for European Sea Bass *Dicentrarchus labrax* L. Reared in Seawater and Freshwater’. *Aquaculture* 231(1–4): 501–515. https://doi.org/10.1016/j.aquaculture.2003.10.020

EUMOFA. 2019a. *The EU Fish Market: 2019 Edition*. Luxembourg: Publications Office of the European Union, 2019.

EUMOFA. 2019b. Case Study *Seabass in the EU*. https://www.intelligrated.com/sites/default/files/pdf/Zappos_Case_Study.pdf.

European Commission. 2017a. ‘Comission Regulation (EU) 2017/293 of 24 May 2017 Amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Protein.’ *Official Journal of the European Union*: 138–192.

European Commission. 2017b. ‘EU Consumer Habits Regarding Fishery and Aquaculture Products - Annex 4 Country Fishes’. (January). https://publications.europa.eu/en/publication-detail/-/publication/a4aa6860-6256-11e7-b2f2-01aa75ed71a1/language-en/format-PDF.

Ewald, N., A. Vidakovic, M. Langeland, A. Kiessling, S. Sampels, & C. Lalander. 2020. ‘Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) – Possibilities and limitations for modification through diet’. *Waste Management* 102: 40–47. https://doi.org/10.1016/j.wasman.2019.10.014.

FAO. 2010. ‘Fats and fatty acids in human nutrition. Report of an Expert Consultation.’ *FAO Food and Nutrition Paper* 91: 1–166. http://www.ncbi.nlm.nih.gov/pubmed/21812367.

FAO. 2013. 'Edible insects. Future prospects for food and feed security*'. 171 FAO Forestry Paper. Food and Agriculture Organization of the United Nations*. ISSN 0258-6150

FAO. 2018. ‘The State of World Fisheries and Aquaculture 2018’. *Meeting the sustainable development goals* 35(3): 227.

FAO. 2020. *The State of World Fisheries and Aquaculture 2020. Sustainability in Action*. Rome.

https://doi.org/10.4060/ca9229en.

Finke, M.D. 2007. ‘Estimate of chitin in raw whole insects’. *Zoo Biology* 26(May 2006): 105–115. https://doi.org/10.1002/zoo.20123

Folch, J., M. Lees, G.S. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J*ournal of Biological Chemistry,* 226(1), 497-509.

Froehlich, H.E., N.S. Jacobsen, T.E. Essington, T. Clavelle, B.S. Halpern. 2018. ‘Avoiding the ecological limits of forage fish for fed aquaculture’. *Nature Sustainability* 1(6): 298–303. http://dx.doi.org/10.1038/s41893-018-0077-1.

Fuentes, A., I. Fernández-Segovia, J.A. Serra, J.M. Barat. 2010. ‘Comparison of wild and cultured Sea Bass (*Dicentrarchus labrax*) quality’. *Food Chemistry* 119(4): 1514–1518. http://dx.doi.org/10.1016/j.foodchem.2009.09.036.

Gasco, L., I. Biasato, S. Dabbou, A. Schiavone, F. Gai. 2019. ‘Animals fed insect-based diets: state-of-the-art on digestibility, performance and product quality’. *Animals* 9(170): 1–32. https://doi.org/10.3390/ani9040170

Gasco, L., F. Gai, G. Maricchiolo, L. Genovese, S. Ragonese, T. Bottari, G. Caruso. 2018. *Feeds for the Aquaculture Sector*. Springer International Publishing. http://link.springer.com/10.1007/978-3-319-77941-6.

Gasco, L., M. Henry, G. Piccolo, S. Marono, F. Gai, M. Renna, C. Lussiana, E. Antonopoulou, P. Mola, S. Chatzifotis. 2016. ‘*Tenebrio molitor* meal in diets for European Sea Bass (*Dicentrarchus labrax L.*) juveniles: growth performance, whole body composition and in vivo apparent digestibility’. *Animal Feed Science and Technology* 220: 34–45. http://dx.doi.org/10.1016/j.anifeedsci.2016.07.003.

Gatlin, D.M., F.T. Barrows, P. Brown, K. Dabrowski, T.G. Gaylord, R.W. Hardy, E. Herman, G. Hu, Å. Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D. Skonberg, E.J. Souza, D. Stone, R. Wilson, E. Wurtele. 2007. ‘Expanding the utilization of sustainable plant products in aquafeeds: A Review’. *Aquaculture Research* 38(6): 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x

Giroud, L., M. Thomas, J.K.K. Tomberlin, A.T.T. Ruiz, L. Yi, J.A. Morales-Ramos, R.L. Jullien, R. Han, J.A. Cortes Ortiz, M.G.G. Rojas, J.A.C. Ortiz, A.T.T. Ruiz, M. Thomas, M.G.G. Rojas, J.K.K. Tomberlin, L. Yi, R. Han. 2016. 'Insects as sustainable food ingredients.

Insect Mass Production Technologies'. *Academic Press*. ISBN: 978-0-12-802856-8

Glencross, B.D. 2009. 'Exploring the nutritional demand for essential fatty acids by aquaculture species'. *Reviews in Aquaculture*, *1*(2), 71-124. https://doi.org/10.1111/j.1753-5131.2009.01006.x

Grau, T., A. Vilcinskas, G. Joop. 2017. 'Sustainable farming of the mealworm *Tenebrio molitor* for the production of food and feed'. *Zeitschrift fur Naturforschung - Section C Journal of Biosciences* 72(9–10): 337–349. https://doi.org/10.1515/znc-2017-0033

Grigorakis, K. 2007. 'Compositional and organoleptic quality of farmed and wild Gilthead Sea Bream (*Sparus aurata*) and Sea Bass (*Dicentrarchus labrax*) and factors affecting it: A Review'. *Aquaculture* 272(1–4): 55–75. http://dx.doi.org/10.1016/j.aquaculture.2007.04.062.

Grigorakis, K. 2017. 'Fillet proximate composition, lipid quality, yields, and organoleptic quality of Mediterranean-farmed marine fish: A Review with Emphasis on New Species'. *Critical Reviews in Food Science and Nutrition*, *57*(14), 2956-2969. https://doi.org/10.1080/10408398.2015.1081145.

Halloran, A., N. Roos, J. Eilenberg, A. Cerutti, & S. Bruun. 2016. 'Life cycle assessment of edible insects for food protein: A review'. *Agronomy for Sustainable Development* 36(4): 1–13. http://dx.doi.org/10.1007/s13593-016-0392-8.

Henry, M., L. Gasco, G. Piccolo, E. Fountoulaki. 2015. 'Review on the use of insects in the diet of farmed fish: past and future'. *Animal Feed Science and Technology* 203(1): 1–22. http://dx.doi.org/10.1016/j.anifeedsci.2015.03.001.

Henry, M.A., L. Gasco, S. Chatzifotis, G. Piccolo. 2018. 'Does dietary insect meal affect the fish immune system? The case of mealworm, *Tenebrio molitor* on European Sea Bass, *Dicentrarchus labrax*'. *Developmental and Comparative Immunology* 81: 204–209. https://doi.org/10.1016/j.dci.2017.12.002.

Hidalgo, F., & E. Alliot. 1988. 'Influence of water temperature on protein requirement and protein utilization in juvenile Sea Bass, *Dicentrarchus labrax*'. *Aquaculture* 72(1–2): 115–129. https://doi.org/10.1016/0044-8486(88)90152-4

Hua, K., J.M. Cobcroft, A. Cole, K. Condon, D.R. Jerry, A. Mangott, C. Praeger, M.J. Vucko, C. Zeng, K. Zenger, & J.M. Strugnell. 2019. 'The future of aquatic protein: implications for protein sources in aquaculture diets'. *One Earth* 1(3): 316–329. https://doi.org/10.1016/j.oneear.2019.10.018.

Iaconisi, V., A. Bonelli, R. Pupino, F. Gai, & G. Parisi. 2018. 'Mealworm as dietary protein source for rainbow trout: body and fillet quality traits'. *Aquaculture* 484: 197–204. https://doi.org/10.1016/j.aquaculture.2017.11.034

Iaconisi, V., S. Marono, G. Parisi, L. Gasco, L. Genovese, G. Maricchiolo, F. Bovera, G. Piccolo. 2017. 'Dietary inclusion of *Tenebrio molitor* larvae meal: effects on growth performance and final quality treats of Blackspot Sea Bream (*Pagellus bogaraveo*)'. *Aquaculture* 476: 49–58. http://dx.doi.org/10.1016/j.aquaculture.2017.04.007.

Ido, A., A. Hashizume, T. Ohta, T. Takahashi, C. Miura, T. Miura. 2019. 'Replacement of fish meal by defatted Yellow Mealworm (*Tenebrio molitor*) larvae in diet improves growth performance and disease resistance in Red Seabream (*Pargus major*)'. *Animals* 9(3). https://doi.org/10.3390/ani9030100

Izquierdo, M.S., A. Obach, L. Arantzamendi, D. Montero, L. Robaina, G. Rosenlund. 2003. 'Dietary lipid sources for Seabream and Seabass: growth performance, tissue composition and flesh quality'. *Aquaculture Nutrition* 9(6): 397–407. https://doi.org/10.1046/j.1365-2095.2003.00270.x

Janssen, C.I.F., A.J. Kiliaan. 2014. 'Long-Chain Polyunsaturated Fatty Acids (LCPUFA) from genesis to senescence: The influence of LCPUFA on neural development, aging, and neurodegeneration'. *Progress in Lipid Research* 53(1): 1–17. http://dx.doi.org/10.1016/j.plipres.2013.10.002.

Janssen, R.H., J.P. Vincken, L.A.M. Van Den Broek, V. Fogliano, C.M.M. Lakemond. 2017. 'nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor, Alphitobius diaperinus*, and *Hermetia illucens*'. *Journal of Agricultural and Food Chemistry* 65(11): 2275–2278. https://doi.org/10.1021/acs.jafc.7b00471

Johnston, I. A. 2008. Improving seafood products for the consumer the biological basis of variability in the texture of fish flesh. *Woodhead Publishing Limited*. http://dx.doi.org/10.1533/9781845694586.5.465.

Johnston, I. A. 1999. 'Muscle development and growth: potential implications for flesh quality in fish'. *Aquaculture* 177(1–4): 99–115. https://doi.org/10.1016/S0044-8486(99)00072-1

Johnston, I. A., X. Li, V.L.A. Vieira, D. Nickell, A. Dingwall, R. Alderson, P. Campbell, R. Bickerdike. 2006. ‘Muscle and flesh quality traits in wild and farmed Atlantic Salmon’. *Aquaculture* 256(1–4): 323–336. https://doi.org/10.1016/j.aquaculture.2006.02.048

Karapanagiotidis, I.T., E. Daskalopoulou, I. Vogiatzis, C. Rumbos, E. Mente, C.G. Athanassiou. 2014. ‘Substitution of fishmeal by fly *Hermetia illucens* prepupae meal in the diet of Gilthead Seabream (*Sparus aurata*)’. *HydroMedit 2014*: 110–114.

Khosravi, S., E. Kim, Y. Lee, S. Lee. 2018. ‘Dietary inclusion of mealworm (*Tenebrio molitor*) meal as an alternative protein source in practical diets for juvenile rockfish (*Sebastes schlegeli*)’. *Entomological Research* 48: 214–221. https://doi.org/10.1111/1748-5967.12306

Kiessling, A., K. Ruohonen, M. Bjørnevik. 2006. ‘Muscle fibre growth and quality in fish’. *Arch. Tierz., Dummerstorf Special Issue* 49: 137–146.

Klasingph, K.C., P.T. S, M.A.L. S, C. Christopher, D. Calvertph, A.K.C. Klasing, P. Thacker, M.A. Lopez, C.C. Calvert, K.C. Klasing, D. Ph, P. Thacker, M.A. Lopez. 2000. ‘Increasing the calcium content of mealworms (*Tenebrio molitor*) To improve their nutritional value for bone mineralization of growing chicks’. *Journal of Zoo and Wildlife Medicine* 31(4): 512–517. https://doi.org/10.1638/1042-7260(2000)031[0512:ITCCOM]2.0.CO;2

Kousoulaki, K., B.-S. Saether, S. Albrektsen, C. Noble. 2015. ‘Review on European Sea Bass (*Dicentrarchus labrax*, Linnaeus, 1758) Nutrition and feed management: a pratical guide for optimizing feed formulation and farming protocols’. *Aquaculture Nutrition* 21: 129–151. https://doi.org/10.1111/anu.12233

Koutsos, L., A. Mccomb, M. Finke. 2019. ‘Insect composition and uses in animal feeding applications: A brief review’. *Annals of the Entomological Society of America* 112(6): 544–551. https://doi.org/10.1093/aesa/saz033

Kris-Etherton, P.M., W.S. Harris, L.J. Appel. 2002. ‘Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease’. *Circulation* 106(21): 2747–2757. https://doi.org/10.1161/01.CIR.0000038493.65177.94

Kroeckel, S., A.G.E. Harjes, I. Roth, H. Katz, S. Wuertz, A. Susenbeth, C. Schulz. 2012. ‘When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute - growth performance and chitin degradation in juvenile turbot (*Psetta maxima*)’. *Aquaculture* 364–365: 345–352.

http://dx.doi.org/10.1016/j.aquaculture.2012.08.041.

Kwasek, K., A.L. Thorne-Lyman, M. Phillips. 2020. 'Can human nutrition be improved through better fish feeding practices? A review paper'. *Critical Reviews in Food Science and Nutrition*: 1–14. https://doi.org/10.1080/10408398.2019.1708698.

Llagostera, Ferrer P., Z. Kallas, L. Reig, D. Amores de Gea. 2019. 'The use of insect meal as a sustainable feeding alternative in aquaculture: current situation, spanish consumers' perceptions and willingness to pay'. *Journal of Cleaner Production* 229: 10–21. https://doi.org/10.1016/j.jclepro.2019.05.012.

Larsen, R., K.E. Eilertsen, E.O. Elvevoll. 2011. 'Health benefits of marine foods and ingredients'. *Biotechnology Advances* 29(5): 508–518. https://doi.org/10.1016/j.biotechadv.2011.05.017

Li, M.H., E.H. Robinson, D.F. Oberle, P. V. Zimba. 2007. 'Effects of various dietary carotenoid pigments on fillet appearance and pigment absorption in channel catfish, *Ictalurus punctatus*'. *Journal of the World Aquaculture Society* 38(4): 557–563. https://doi.org/10.1111/j.1749-7345.2007.00130.x

Lichtenstein, A.H., L.J. Appel, M. Brands, M. Carnethon, S. Daniels, H.A. Franch, B. Franklin, P. Kris-Etherton, W.S. Harris, B. Howard, N. Karanja, M. Lefevre, L. Rudel, F. Sacks, L. Van Horn, M. Winston, J. Wylie-Rosett. 2006. 'Diet and lifestyle recommendations Revision 2006: A Scientific Statement from the American Heart Association Nutrition Committee'. *Circulation* 114(1): 82–96. https://doi.org/10.1161/CIRCULATIONAHA.106.176158

Listrat, A., B. Lebret, I. Louveau, T. Astruc, M. Bonnet, L. Lefaucheur, B. Picard, J. Bugeon. 2016. 'How muscle structure and composition influence meat and flesh quality'. *Scientific World Journal* 2016. https://doi.org/10.1155/2016/3182746

López-Albors, O., I. Abdel, M.J. Periago, M.D. Ayala, A.G. Alcázar, C.M. Graciá, C. Nathanailides, J.M. Vázquez. 2008. 'Temperature influence on the white muscle growth dynamics of the Sea Bass *Dicentrarchus labrax*, L. flesh quality implications at commercial size'. *Aquaculture* 277(1–2): 39–51. https://doi.org/10.1016/j.aquaculture.2008.02.015

Lund, E.K. 2013. 'Health benefits of seafood; is it just the fatty acids?' *Food Chemistry* 140(3): 413–420. http://dx.doi.org/10.1016/j.foodchem.2013.01.034.

Lupatsch, I., G.W. Kissil, D. Sklan. 2001. ‘Optimization of feeding regimes for European Sea Bass *Dicentrarchus labrax*: a factorial approach’. *Aquaculture* 202(3–4): 289–302. https://doi.org/10.1016/S0044-8486(01)00779-7

Magalhães, R., A. Sánchez-López, R.S. Leal, S. Martínez-Llorens, A. Oliva-Teles, H. Peres. 2017. ‘Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*)’. *Aquaculture* 476: 79–85. http://dx.doi.org/10.1016/j.aquaculture.2017.04.021.

Makkar, H.P.S., G. Tran, V. Heuzé, P. Ankers. 2014. ‘State of the art on use of insects as animal feed’. *Animal Feed Science and Technology* 197: 1–33. http://dx.doi.org/10.1016/j.anifeedsci.2014.07.008.

Makol, A., S. Torrecillas, M.J. Caballero, A. Fernández-Vaquero, M.S. Izquierdo. 2012. ‘Effect of long term feeding with conjugated linoleic acid (CLA) in growth performance and lipid metabolism of European sea bass (*Dicentrarchus labrax*)’. *Aquaculture* 368–369: 129–137. http://dx.doi.org/10.1016/j.aquaculture.2012.09.015.

Makol, Alex, S. Torrecillas, A. Fernández-vaquero, L. Robaina, D. Montero, M. José, L. Tort, M. Izquierdo, A.R.A. Dha. 2009. ‘Comparative biochemistry and physiology , part b effect of conjugated linoleic acid on dietary lipids utilization , liver morphology and selected immune parameters in sea bass juveniles (*Dicentrarchus labrax*)’. *Comparative Biochemistry and Physiology, Part B* 154(2): 179–187. http://dx.doi.org/10.1016/j.cbpb.2009.06.001.

Mancini, S., I. Medina, V. Iaconisi, F. Gai, A. Basto, G. Parisi. 2018. ‘Impact of black soldier fly larvae meal on the chemical and nutritional characteristics of rainbow trout fillets’. *Animal* 12(8): 1672–1681. doi:10.1017/S1751731117003421

Mancuso, T., L. Baldi, L. Gasco. 2016. ‘An empirical study on consumer acceptance of farmed fish fed on insect meals: the italian case’. *Aquaculture International* 24(5): 1489–1507. https://doi.org/10.1007/s10499-016-0007-z

Mansano, C.F.M., T.M.T. do Nascimento, H. Peres, F.H.F. Rodrigues, K.U. Khan, R.S. Romaneli, N.K. Sakomura, J.B.K. Fernandes. 2020. ‘Determination of the optimum dietary essential amino acid profile for growing phase of Nile Tilapia by deletion method’. *Aquaculture* 523: 735204. https://doi.org/10.1016/j.aquaculture.2020.735204.

Mariod, A.A. 2020. 'African edible insects as alternative source of food, oil, protein and bioactive components' *Springer Nature*.

Martinsdóttir, E. 2010. 'Sensory analysis for food and beverage quality control'. *Sensory Quality Management of Fish* cap.10, pp: 293-315. http://dx.doi.org/10.1533/9781845699512.3.293.

Mastoraki, M., P. Mollá Ferrándiz, S.C. Vardali, D.C. Kontodimas, Y.P. Kotzamanis, L. Gasco, S. Chatzifotis, E. Antonopoulou. 2020. 'A comparative study on the effect of fish meal substitution with three different insect meals on growth, body composition and metabolism of European sea bass (*Dicentrarchus labrax L.*)'. *Aquaculture* 528: 735511. https://doi.org/10.1016/j.aquaculture.2020.735511.

Mikołajczak, Z., M. Rawski, J. Mazurkiewicz, B. Kieronczyk, D. Józefiak. 2020. 'The effect of hydrolyzed insect meals in sea trout fingerling (*Salmo trutta m. trutta*) Diets on growth performance, microbiota and biochemical blood parameters'. *Animals* 10: 1–20. https://doi.org/10.3390/ani10061031

Morales, L.E., A. Higuchi. 2018. 'Is fish worth more than meat? – How consumers' beliefs about health and nutrition affect their willingness to pay more for fish than meat'. *Food Quality and Preference* 65: 101–109. https://doi.org/10.1016/j.foodqual.2017.11.004.

Mozaffarian, D., J.H.Y. Wu. 2012. '(N-3) Fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary?' *The Journal of Nutrition* 142(3): 614S-625S.

Ng, W.K. 2001. 'Potential of mealworm (*Tenebrio molifor*) as an alternative protein source in practical diets for African Catfish, *Clarias gariepinus*'. *Aquaculture Research* 32(SUPPL. 1): 273–280. https://doi.org/10.1046/j.1355-557x.2001.00024.x

Nogales-Mérida, S., P. Gobbi, D. Józefiak, J. Mazurkiewicz, K. Dudek, M. Rawski, B. Kierończyk, A. Józefiak. 2019. 'Insect meals in fish nutrition'. *Reviews in Aquaculture* 11(4): 1080–1103. https://doi.org/10.1111/raq.12281

NRC. 2011. *Nutrient Requirements of Fish and Shrimp*. The National Academic Press, Washington DC.

Parodi, A., A. Leip, I.J.M. De Boer, P.M. Slegers, F. Ziegler, E.H.M. Temme, M. Herrero, H. Tuomisto, H. Valin, C.E. Van Middelaar, J.J.A. Van Loon, H.H.E. Van Zanten. 2018. 'The potential of future foods for sustainable and healthy diets'. *Nature Sustainability* 1(12): 782–789. http://dx.doi.org/10.1038/s41893-018-0189-7.

Paul, A., M. Frederich, R.C. Megido, T. Alabi, P. Malik, R. Uyttenbroeck, F. Francis, C. Blecker, E. Haubruge, G. Lognay, S. Danthine. 2017. ‘Insect fatty acids: a comparison of lipids from three orthopterans and *Tenebrio molitor* L. larvae’. *Journal of Asia-Pacific Entomology* 20(2): 337–340. https://doi.org/10.1016/j.aspen.2017.02.001

Peres, H., A. Oliva-Teles. 1999. ‘Effect of dietary lipid level on growth performance and feed utilization by European sea bass Juveniles (*Dicentrarchus labrax*)’. *Aquaculture* 179(1–4): 325–334. https://doi.org/10.1016/S0044-8486(99)00168-4

Peres, H., A. Oliva-Teles. 2007. ‘Effect of the dietary essential amino acid pattern on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*)’. *Aquaculture* 267(1–4): 119–128. https://doi.org/10.1016/j.aquaculture.2007.01.010

Periago, M.J., M.D. Ayala, O. López-Albors, I. Abdel, C. Martínez, A. García-Alcázar, G. Ros, F. Gil. 2005. ‘Muscle cellularity and flesh quality of wild and farmed sea bass, *Dicentrarchus labrax* L.’ *Aquaculture* 249(1–4): 175–188. https://doi.org/10.1016/j.aquaculture.2005.02.047

Piccolo, G., V. Iaconisi, S. Marono, L. Gasco, R. Loponte, S. Nizza, F. Bovera, G. Parisi. 2017. ‘Effect of *Tenebrio molitor* larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of Gilthead sea bream (*Sparus aurata*)’. *Animal Feed Science and Technology* 226: 12–20. http://dx.doi.org/10.1016/j.anifeedsci.2017.02.007.

Pieniak, Z., F. Vanhonacker, W. Verbeke. 2013. ‘Consumer knowledge and use of information about fish and aquaculture’. *Food Policy* 40: 25–30. http://dx.doi.org/10.1016/j.foodpol.2013.01.005.

Rema, P., S. Saravanan, B. Armenjon, C. Motte, J. Dias. 2019. ‘Graded incorporation of defatted yellow mealworm (*Tenebrio molitor*) in Rainbow Trout (*Oncorhynchus mykiss*) Diet improves growth performance and nutrient retention’. *Animals* 9(4). https://doi.org/10.3390/ani9040187

Renna, M., A. Schiavone, F. Gai, S. Dabbou, C. Lussiana, V. Malfatto, M. Prearo, M.T. Capucchio, I. Biasato, E. Biasibetti, M. De Marco, A. Brugiapaglia, I. Zoccarato, L. Gasco. 2017. ‘Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) Larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss (*Walbaum*))* diets’. *Journal of Animal Science and Biotechnology* 8(1): 1–13. https://doi.org/10.1186/s40104-

017-0191-3

Ruiz-Chico, J., A.R. Peña-Sánchez, J.M. Biedma-Ferrer, M. Jiménez-García. 2020. 'Social acceptance of aquaculture in andalusian atlantic coast (Spain): An emerging economy sector'. *Foods* 9(7): 1–16. https://doi.org/10.3390/foods9070910

Rumpold, B.A., O.K. Schlüter. 2013. 'Potential and challenges of insects as an innovative source for food and feed production'. *Innovative Food Science and Emerging Technologies* 17: 1–11. http://dx.doi.org/10.1016/j.ifset.2012.11.005.

Sánchez-Muros, M.J., F.G. Barroso, C. de Haro. 2016. Insects as sustainable food ingredients brief summary of insect usage as an industrial animal feed/feed ingredient. *Elsevier Inc*. http://dx.doi.org/10.1016/B978-0-12-802856-8/00010-7.

Sánchez-muros, M.J., F.G. Barroso, F. Manzano-agugliaro. 2014. 'Insect meal as renewable source of food for animal feeding: a review'. *Journal of Cleaner Production* 65: 16–27. http://dx.doi.org/10.1016/j.jclepro.2013.11.068.

Sardesai, V.M. 2020. 'Integrative and functional medical nutrition therapy'. *Integrative and Functional Medical Nutrition Therapy*: 135–149. https://doi.org/10.1007/978-3-030-30730-1_10

Shepherd, C.J., A.J. Jackson. 2013. 'Global fishmeal and fish-oil supply: inputs, outputs and markets'. *Journal of Fish Biology* 83(4): 1046–1066. https://doi.org/10.1111/jfb.12224

Skalli, A., J.H. Robin, N. Le Bayon, H. Le Delliou, J. Person-Le Ruyet. 2006. 'Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (*Dicentrarchus labrax*)'. *Aquaculture* 255(1–4): 223–232. https://doi.org/10.1016/j.aquaculture.2005.12.006

Sogari, G., M. Amato, I. Biasato, S. Chiesa, L. Gasco. 2019. 'The potential role of insects as feed: A Multi-Perspective Review'. *Animals* 9(4): 1–15. https://doi.org/10.3390/ani9040119

Spence, C., C.A. Levitan, M.U. Shankar, M. Zampini. 2010. 'Does food color influence taste and flavor perception in humans?' *Chemosensory Perception* 3(1): 68–84. https://doi.org/10.1007/s12078-010-9067-z

Spranghers, T., M. Ottoboni, C. Klootwijk, A. Ovyn, S. Deboosere, B. De Meulenaer, J. Michiels, M. Eeckhout, P. De Clercq, S. De Smet. 2017. 'Nutritional composition of black soldier fly (*Hermetia illucens*) Prepupae reared on different organic waste substrates'. *Journal of the Science of Food and Agriculture* 97(8): 2594–2600. https://doi.org/10.1002/jsfa.8081

St-Hilaire, S., C. Sheppard, J.K. Tomberlin, S. Irving, L. Newton, M.A. McGuire, E.E. Mosley, R.W. Hardy, & W. Sealey. 2007. 'Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*'. *Journal of the World Aquaculture Society* 38(1): 59–67. https://doi.org/10.1111/j.1749-7345.2006.00073.x

Stadtlander, T., A. Stamer, A. Buser, J. Wohlfahrt, F. Leiber, C. Sandrock. 2017. '*Hermetia illucens* meal as fish meal replacement for rainbow trout on farm abstract'. *Journal of Insects as Food and Feed* 3(3): 165–175. https://doi.org/10.3920/JIFF2016.0056

Suman, S.P., P. Joseph. 2013. 'Myoglobin chemistry and meat color'. *Annual Review of Food Science and Technology* 4(1): 79–99. https://doi.org/10.1146/annurev-food-030212-182623

Swanson, D., R. Block, S.A. Mousa. 2012. 'Omega-3 fatty acids EPA and DHA: health benefits throughout life'. *Advances in Nutrition* 3(1): 1–7. https://doi.org/10.3945/an.111.000893

Tacon, A.G.J., M.R. Hasan, M. Metian. 2011. 'Demand and supply of feed ingredients for farmed fish and crustaceans : trends and prospects'. *FAO Fisheries and Aquaculture Technical Paper* 564: 87. http://www.fao.org/docrep/015/ba0002e/ba0002e.pdf.

Tacon, A.G.J., M. Metian. 2013. 'Fish matters: importance of aquatic foods in human nutrition and global food supply'. *Reviews in Fisheries Science* 21(1): 22–38. https://doi.org/10.1080/10641262.2012.753405

Tang, C., D. Yang, H. Liao, H. Sun, C. Liu, L. Wei, F. Li. 2019. 'Edible insects as a food source : A Review'. 1: 1–13. https://doi.org/10.1186/s43014-019-0008-1

Taylor, R.G., S.O. Fjaera, P.O. Skjervold. 2002. 'Salmon fillet texture is determined by myofiber-myofiber and myofiber-myocommata attachment'. *Journal of Food Science* 67(6): 2067–2071. https://doi.org/10.1111/j.1365-2621.2002.tb09502.x

Thong, N.T., H.S. Solgaard. 2017. 'Consumer's food motives and seafood consumption'. *Food Quality and Preference*, 56: 181–188. https://doi.org/10.1016/j.foodqual.2016.10.008

Tibaldi, E., S.J. Kaushik. 2005. 'Amino acid requirements of Mediterranean fish species'. *Mediterranean fish nutrition Zaragoza: CIHEAM, 2005. p. 59-65 (Cahiers Options*

Méditerranéennes; n. 63). http://om.ciheam.org/article.php?IDPDF=5600066

Tinder, A.C., R.T. Puckett, N.D. Turner, J.A. Cammack, J.K. Tomberlin. 2017. ‘Bioconversion of sorghum and cowpea by black soldier fly (*Hermetia illucens* (L.)) Larvae for alternative protein production’. *Journal of Insects as Food and Feed* 3(2): 121–130. https://doi.org/10.3920/JIFF2016.0048

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

Tocher, D.R. 2015. ‘Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective’. *Aquaculture* 449: 94–107. http://dx.doi.org/10.1016/j.aquaculture.2015.01.010.

Torgersen, J.S., E.O. Koppang, L.H. Stien, A. Kohler, M.E. Pedersen, T. Mørkøre. 2014. ‘Soft texture of atlantic salmon fillets is associated with glycogen accumulation’. *PLoS ONE* 9(1). https://doi.org/10.1371/journal.pone.0085551

Turchini, G.M., T.O. Eroldog, K. Engin, M. Arslan, N.A. Sirkeciog. 2013. ‘Fatty acid metabolism in European sea bass (*Dicentrarchus labrax*): Effects of n-6 PUFA and MUFA in fish oil replaced diets’. *Fish Physiology and Biochemistry*, *39*(4): 941–955. doi: 10.1007/s10695-012-9753-7

Valente, L.M.P., K.A. Moutou, L.E.C. Conceição, S. Engrola, J.M.O. Fernandes, I.A. Johnston. 2013. ‘What determines growth potential and juvenile quality of farmed fish species?’ *Reviews in Aquaculture* 5(SUPPL.1). https://doi.org/10.1111/raq.12020

Valente, L.M.P., P. Rema, V. Ferraro, M. Pintado, I. Sousa-Pinto, L.M. Cunha, M.B. Oliveira, M. Araújo. 2015. ‘Iodine enrichment of rainbow trout flesh by dietary supplementation with the red seaweed *Gracilaria vermiculophylla*’. *Aquaculture* 446: 132–139. http://dx.doi.org/10.1016/j.aquaculture.2015.05.004.

Van Huis, Arnold. 2020. ‘Insects as food and feed, a new emerging agricultural sector: A Review’. *Journal of Insects as Food and Feed* 6(1): 27–44. https://doi.org/10.3920/JIFF2019.0017

Van Huis, Arnold. 2013. ‘Potential of insects as food and feed in assuring food security’. *Annual Review of Entomology* 58(1): 563–583. https://doi.org/10.1146/annurev-ento-120811-153704

Van Huis, Arnold, D.G.A.B. Oonincx. 2017. ‘The environmental sustainability of insects as food and feed. A Review’. *Agronomy for Sustainable Development* 37(5). https://doi.org/10.1007/s13593-017-0452-8

Varelas, V. 2019. ‘Food wastes as a potential new source for edible insect mass production for food and feed: A Review’. *Fermentation* 5(3). https://doi.org/10.3390/fermentation5030081

Veggetti, A., F. Mascarello, P.A. Scapolo, A. Rowlerson. 1990. ‘Hyperplastic and hypertrophic growth of lateral muscle in *Dicentrarchus labrax* (L.) - An ultrastructural and morphometric study’. *Anatomy and Embryology* 182(1): 1–10.

Videler, J.J. 2011. ‘An opinion paper: Emphasis on white muscle development and growth to improve farmed fish flesh quality’. *Fish Physiology and Biochemistry* 37(2): 337–343. https://doi.org/10.1007/s10695-011-9501-4

Wang, J., Q. Tao, Z. Wang, K. Mai, W. Xu, Y. Zhang, Q. Ai. 2017. ‘Effects of fish meal replacement by soybean meal with supplementation of functional compound additives on intestinal morphology and microbiome of Japanese Seabass (*Lateolabrax japonicus*).' *Aquaculture Research 48*(5), 2186-2197. https://doi.org/10.1111/are.13055

Wang, Y.S., M. Shelomi. 2017. ‘Review of black soldier fly (*Hermetia illucens*) as animal feed and human food’. *Foods* 6(10): 91. https://doi.org/10.3390/foods6100091

Weil, C., F. Lefèvre, J. Bugeon. 2013. ‘Characteristics and metabolism of different adipose tissues in fish’. *Reviews in Fish Biology and Fisheries* 23(2): 157–173. doi: 10.1007/s11160-012-9288-0

Weindl, I., A. Popp, B.L. Bodirsky, S. Rolinski, H. Lotze-Campen, A. Biewald, F. Humpenöder, J.P. Dietrich, M. Stevanović. 2017. ‘Livestock and human use of land: productivity trends and dietary choices as drivers of future land and carbon dynamics’. *Global and Planetary Change* 159(August): 1–10. https://doi.org/10.1016/j.gloplacha.2017.10.002

Wilson, R.P. 1986. ‘Protein and amino acid requirements of fishes’. *Ann. Rev. Nutr.,* 6: 225–244.