Potential of prepupae meal of Black Soldier Fly (*Hermetia illucens*) as fish meal substitute in European sea bass juveniles (*Dicentrarchus labrax*): Implication in flesh quality

Rita da Cunha Pedrosa
Mestrado em Recursos Biológicos Aquáticos
Departamento de Biologia
2016

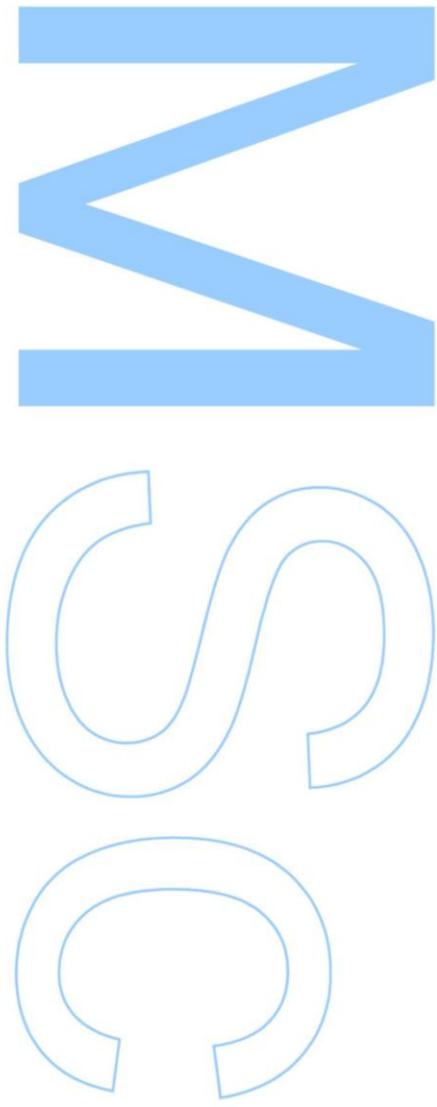
Orientador
Doutora Maria Helena Peres, investigadora auxiliar CIIMAR

Coorientador
Doutora Giuliana Parisi, professora associada DISPAA, Universidade de Florença

Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, _____/_____/_____



Acknowledgments

I would like to express my sincere gratitude towards Dra. Helena Peres for all her patient knowledge and dedication during the writing of this thesis and especially for getting me the opportunity to learn new skills by doing my master thesis abroad, because of this I was able to fulfill one of my dreams.

I also want to express my gratitude to Prof. Giuliana Parisi, for accepting me in her lab, for all her support, attention and care. For not only helping me developing my thesis but always worrying for my well-being.

A very special thank you for all the researchers and technicians working at DISPAA (Department of Agri-Food Production and Environmental Sciences), for all the help attention and kindness even though we did not speak the same languages we always ended up understanding each other. A special thank you to Monica Borgogno and Valeria Iaconisi for the tremendous support, kindness and for all the hours spend in the lab helping me. This was essential for ending my work on time.

Also, I would like to show my appreciation to my family and friends. To my mother for all her advices, unconditional love and strength that helped me through not only my master thesis but all my life. She is a big part of why I got so far. To Sílvia and Vítor, who had a very important role in this thesis, thank you for all the kind words and for always being available to help me. To Eva and Isabella people that I know I can always count on, thank you for the special friendship that helped and teached me so much. To Rafael for all the love, laughs and for always pushing me further and never letting me give up.

Abstract

Aquaculture has become the solution for the increasing demand of fish by the human population. The main protein source used in fish diets has been fish meal (FM) due to its numerous nutritional advantages. Still due to overfishing there has been a shortage of FM. Insect meal, as an alternative protein source, is not a new subject in fish nutrition and is very suitable as an alternative to FM due to its similar nutritional profile. There have been studies regarding the substitution of fish meal with insect meal in various fish species, with promising results.

When dealing with food products, it is very important to insure food safety. So there is the need to be certain that the new ingredient on the diets does not affect fish quality, for this may have a great impact on consumers choice and human health. For this reason, there is the need to evaluate quality parameters such as colour, pH, fatty acid (FA) profile, lipid oxidation among others so that the products can safely enter the market. without compromising colour, liver FA profile and nutritional index. In this study it was verified that *Hermetia* meal (HM) incorporation in diets lead to no significant differences in colour, liver FA profile and nutritional index. Also, these diets may lead to a decrease of lipid oxidation due to a significant decrease of TBARS values in the muscle of fish fed HM diets. Although studies report differences in muscle FA profile of fish fed HM diets in this study these differences were not so significant. Sensory analysis, evaluation of lipid oxidation and fillet FA profile may bring further insights to evaluate the quality of fish fed this alternative protein source.

Keywords: Insect meal, black soldier fly, *Dicentrarchus labrax*, European seabass, flesh quality parameters, shelf life

Resumo

Aquacultura tornou se a solução á crescente procura de peixe pela população humana A principal fonte de proteína das dietas usadas em peixe tem sido a farinha de peixe devido as usas inúmeras vantagens nutricionais. Contudo, devido a sobrepesca tem havido uma escassez da farinha de peixe. Farinha de inseto, como fonte alternativa de proteína não é um novo assunto na área de nutrição de peixes e é considerado uma boa alternativa a farinha de peixe uma vez que tem um perfil nutricional semelhante. Existem estudos que acerca da substituição da farinha de peixe por farinha de inseto e várias espécies de peixes que revelam resultados promissores.

Ao lidar com produtos alimentares, é muito importante assegurar a segurança alimentar. Por isso é necessário ter a certeza que o novo ingrediente da dieta não afeta a qualidade do peixe, pois isto poderá ter um grande impacto na escolha do consumidor e na saúde humana. Esta é a razão pela qual é necessário avaliar parâmetros de qualidade tais como cor, ph, perfil de ácidos gordos e índices nutricionais. Neste estudo verificou-se que a incorporação de farinha de *Hermetia* nas dietas não levou a diferenças na cor, no perfil de ácidos gordos do fígado e nos índices nutricionais. Também, estas dietas possivelmente levaram a uma diminuição da oxidação lipídica devido a diminuição significativa dos valores de TBARS no músculo de peixes alimentas com dietas com farinha de *Hermetia.* Apesar de alguns estudos relatarem diferenças no perfil de ácidos gordos do músculo de peixes alimentados com dietas com farinha de *Hermetia,* neste estudo as diferenças não foram significativas. Análises sensoriais, avaliação da oxidação lipídica e do perfil de ácidos gordos dos filetes podem trazer mais informações para avaliar a qualidade de peixes alimentados com esta fonte alternativa de proteína.

Palavras-chave: farinha de inseto, *Hermetia illucens*, mosca soldado negro, *Dicentrarchus labrax,* robalo, parâmetros de qualidade

Table of contents

List of figures

List of tables

List of abbreviations

AA: Amino acids

AI: Atherogenic index

ARA: Arachidonic acid

BSF: Black soldier fly

DHA: Docosahexaenoic acid

EAA: Essential amino acid

EPA: Eicosapentaenoic acid

FA: Fatty acids

FM: Fish meal

HH: hypocholesterolaemic/ hypercholesterolaemic ratio

HM: *Hermetia* meal

HUFA: Highly unsaturated fatty acids

LC-PUFA: Long chain polyunsaturated fatty acids

MDA: Malondialdehyde

MUFA: Monounsaturated fatty acids

NEAA: Non-essential amino acids

PUFA: Polyunsaturated fatty acid

SFA: Saturated fatty acids

TBARS: Thiobarbituric acid reactive substances

TI: Thrombogenic index

Introduction

1. Aquaculture

In many countries, marine living resources are an important and growing source of protein. Recent records show that from the 1960 to 2014, the world apparent fish consumption per capita increased from an average of 9.9 kg to 20.0 kg per year. Considering that overexploitation has led to a worldwide decrease of natural fish stocks, aquaculture is a global solution, providing a sustainable seafood resources (Basurco *et al.,* 2011; FAO, 2016).

Aquaculture is described as the farming of aquatic organisms using human intervention in order to improve the production of aquatic species. Its early history and nature is unclear; records show that in 2500 B.C. and 1100 B.C. aquaculture was already present in Egypt and China, respectively. At the beginning, aquaculture fish was produced under traditional small extensive units with low density stocking and minimal input. However, with the world's population growth in 20th century and the decrease of natural fish stocks, aquaculture systems have undergone a significant intensification of production, through a technology-driven processes, to meet the request of fishery products by the consumers (White *et al.,* 2004; Sapkota *et al.,* 2008. Nowadays, aquaculture remains one of the fastest-growing sector of the food production systems (FAO, 2016).

Aquatic organisms represent an important protein source for the human population. In 2014, fish consumption represented nearly 20% of average animal protein intake per capita (FAO, 2016). It is expected an increase of this share of aquaculture production for human consumption, due to an increased world demand for aquatic foods. Indeed, aquaculture is now recognized as the only way to meet the increasing demands for aquatic foods, as by 2050, the world's population is expected to reach the 9.7 billion people (Nkafamiya *et al.,* 2007; FAO, 2016). Besides, the increase of knowledge and consciousness of the nutritional benefits of fish consumption on human health, as also been contributed for the fish-food intake per capita (Nkafamiya *et al.,* 2007; Watters *et al.,* 2012).

During the last four decades, it was observed an extreme improvement on aquafeed formulation and production technology as well as on feed management practices. However, in order to attain the economic, environmental and social challenges of aquaculture production it is required further research. Formulation issues, especially the demand for aquafeeds that meet the nutritional requirements of aquaculture fish species at different life stages, are of major importance (FAO, 2016).

To maximize aquaculture production and to obtain a secure and excellent fish-food product it is necessary a good feed quality, which needs to be monitored constantly. Supervising the composition of aquafeeds and other inputs provides the production of healthy fish with optimal nutritional composition (FAO, 2014). Therefore aquaculture is capable to ensure a food product with elevated and constant nutrient composition.

2. Aquaculture production of European bass

European sea bass (*Dicentrarchus labrax)* (Fig. 1) belongs to the Moronidae family, is an eurythermic (5-28 °C) and euryhaline (3‰ to full strength sea water) species. It possesses the ability to inhabit coastal inshore waters, and occurs in estuaries and brackish water lagoons. Annual spawning depends on the population; on the Mediterranean population the spawning period goes from December to March, and in the Atlantic population its early spring. As predators, sea bass feeding habits include small fish, prawns, crabs and cuttlefish (FAO, 2015).

Figure1 European seabass (*Dicentrarchus labrax)*. Source: Fish Base. http://www.fishbase.org

Mediterranean sea bass aquaculture industry represents one of the major cases of success in European aquaculture, in the last decade (Fig. 2). Sea bass is not only one of the most important finfish species cultured in the Mediterranean region but it is also the first marine non-salmonid species to be commercially produced in Europe. Its overall quality and nutritional traits, namely its lean appearance (lipids deposition is located mainly in liver) and high quality lipids, rich in polyunsaturated fatty acid (PUFA), have led to an increased demand for this fish species in Europe (Poli *et al.,* 2001).

Currently, it production has been experienced a rapid expansion in Greece, Turkey, Italy, Spain, Croatia and Egypt which are the top producers (Oliva Teles, 2000; Kroeckel *et al.,* 2012; FAO, 2015).

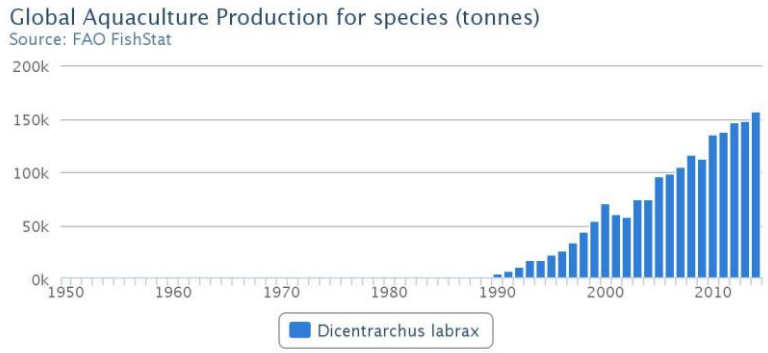


Figure 2. Global Aquaculture Production of European sea bass (tones) source: FAO, 2015.

As carnivorous species, European sea bass has a high protein requirement. Optimum dietary protein levels depend on the life stage. For juvenile sea bass, optimum dietary crude protein level was estimated to be 50 to 45%, in diets with 17 to 20 MJ/kg of digestible energy and is not affected by water temperature (Oliva Teles, 2000; Kousoulaki *et al*., 2015). This balance between dietary total protein and digestible energy is important to maximize growth, nutrient utilization efficiency at the lower cost. Excess of protein will have both economic and environmental consequences, as the expensive protein supplier will be used for energy purpose (Kaushik, 1998; Cho and Bureau, 2001). Also, the excess of digestible energy may limit the voluntary feed intake and growth (Peres and Oliva-Teles, 1999)

Sea bass requires the same ten EAA as the salmonids and the established EAA requirements levels (except for methionine; Thebault *et al*., 1985) are lower than that of fish meal (FM) (Kousoulaki *et. al*., 2015). However, it was observed that best overall growth and feed utilization of sea bass juveniles were obtained with a dietary AA pattern of high-quality FM, rather than with the ideal protein AA pattern of this specie (Peres and Oliva-Teles 2007). Moreover, Peres and Oliva-Teles (2006) suggested an optimum EAA to non-essential amino acid (NEAA) (EAA/NEAA) ratio of 50/50 or 60/40 for high growth performance and for effective protein and energy utilization of sea bass juveniles.

Dietary lipids level is especially important regarding carcass quality. The optimum lipid level was established to be 11 to 26% of the diet, depending on the life stage. For juveniles, it was observed that the increase of dietary lipid from 12 to 24% led to an increase of protein efficiency ratio and energy retention (Peres and Oliva-Teles, 1999). However, the increase of dietary lipid levels may result in a higher lipids deposition that may have some impact on the nutritional value of fillets and storage time of the fish.

Like other carnivorous marine fish, sea bass lacks the capacity to elongate and desaturate C18 fatty acid (FA) to eicosapentaenoic acid and docosahexaenoic acid (EPA and DHA respectively), and then to arachidonic acid (ARA). Consequently, these long chain polyunsaturated fatty acids (LC-PUFA) are considered as essential FAs and sea bass must obtain it through the diet (Mourente and Tocher, 1993; Mourente and Dick, 2002; NRC, 2011).

From the consumer perspective, since this specie is known for its high levels of LC-PUFA it is necessary to assure the maintenance of sea bass FA profile to ensure the best quality of the final product through the fish diet composition.

3. Searching for an Alternative Protein Source

To ensure nutritious diets, aquafeed has been relying on the use of FM and fish oils as the optimal protein and lipids sources. Indeed, the unique nutritional proprieties of both FM and fish oil have caused an increased demand of these prime ingredients for aquafeeds. However, due to the intense global search for FM and consequently decreased availability and increased price, its extensive utilization in aquafeeds has become an unbearable situation. Besides the economic issue, as FM prices continued to increase, social and environmental issues have also been raised, as almost 30% of total fish catch is transformed in FM and fish oil for use in livestock and fish feeds. Overall, the price increase of FM has significantly affected the aquaculture cost production, making this feedstuff less accessible to small farmers (FAO, 2009; FAO, 2012; Barroso *et al.*, 2014; FAO, 2016). In fact, in a commercial aquaculture operation, feeds represent 40-70% of the cost of fish produced and this percentage is particularly high in the case of aquaculture of carnivorous species.

The objective to produce fish while saving marine resources has prompted feed producers to decrease the use of FM and fish oil as ingredients of the formulated feeds, in the last years. However, for many fish species, particularly for those with carnivorous feeding habits, it is still required a FM inclusion rate of 15-30% (Nordahl, 2011; Henry *et al.,* 2015). For these species, dietary incorporation of FM ensures maximization of feed efficiency, fish growth, and fish health, due to its superior nutritional quality, digestibility and palatability.

FM is a brown powder produced after processing fresh raw fish or trimmings from food fish. It consists of 60 to 72% protein, 5 to 12 % lipids including a high level of LC-PUFA, EPA and DHA. Therefore, there is a special interest in alternative protein sources with

nutritional characteristics similar to FM, in terms of protein, amino acids (AA), phospholipids, FAs (EPA and DHA) and mineral contents. The dietary replacement of FM by these alternative ingredients would allow aquaculture production to promote optimum fish development, growth and reproduction and continue to be an economical and sustainable productive process (Nordahl*,* 2011; Barroso *et al.,* 2014).

Researchers have been motivated to study new protein sources. The combination of research advances in terms of fish nutritional aspects and feed production technology have opened the possibility of partial or total replacement of FM by alternative protein sources. The potential of FM replacement by plant protein sources have been largely studied in the last decades. Although some vegetable products such as soybean, rapeseed, corn and gluten possess high/moderate protein content, they may present several drawbacks including presence of anti-nutrients factors, high levels of fiber, starch, especially non-soluble carbohydrates, as well as an unbalanced EAA profile, low digestibility and unfavorable palatability (Gatlin *et al.*, 2007). Due to these constraints, the high inclusion levels of some plant feedstuffs may decrease the feed palatability, reduce voluntary feed intake and growth performance plus cause inflammation of the digestive tract (Gatlin *et al*., 2007). Other novel ingredients developed through various processing technologies, as the agro-industrial by-products, have been studied. This is the case of dried distillers grains with solubles (DDGS) and algae obtained from the ethanol industry. Biological enhancement of low-nutritional quality feedstuffs have also been studied, involving the use of microorganisms through yeast, bacterial and fungal fermentations to modify the chemical composition of the original ingredient (Ayoola, 2010).

Since fish are monogastric species, they possess particular nutritional needs in terms of high quality and quantity of protein in feeds (Sànchez-Muros *et al.,* 2014; Henry *et al.,* 2015). Thus, for the majority of the fish species, despite the progress made in reducing FM inclusion in diets by the use of other protein sources adequately supplemented with limiting EAA and with low levels of anti-nutritional factors, available data show a 10% growth in production of high trophic level species, that still rely on FM to supply part of dietary protein (Torstensen *et al.,* 2008).

4. Insect meal

Insects are part of the natural diet of both freshwater and marine fish, and because they are rich in AA, lipids, vitamins and minerals and leave a small ecological footprint (no need for arable land, low need for energy and water), insect meal has been

considered as potential alternatives to FM (Henry *et al.*, 2015). More recently, the idea to incorporate insect meals into aquafeed has been brought to the spotlight, although this idea has been around for almost 40 years (van Huis, 2013; Barroso *et al.,* 2014).

Insect larvae have a fast growth, transforming low quality organic waste into good and the final product is a very efficient bioconversion of manure is an abundant amount of insect larvae or prepupae, rich in proteins and lipids (Newton *et al.*, 2004 Henry *et al.*, 2015). Moreover, insects have fast reproduction, need a small breeding area and have high feed conversion efficiency which bring different environmental and economical benefits of using insect meal (Klunder *et al.,* 2012; Barroso *et al.,* 2014).

In recent studies, this innovative ingredient has been used in the form of insect meal in diets, and experimented in feeding of many fish species. Results have shown that, depending on the fish and insect species, there is a reduction of growth performance if insect meal is incorporated at levels higher than 30%. Continuous research and improvement of the nutritional quality of insect meal as well as the continue pressure to reduce FM in aquafeeds pointed out insect meal as a good candidate as a potential alternative to FM in aquafeeds (van Huis *et al*, 2013; Barroso *et al.,* 2014; Tran *et al.,* 2015).

An up most important aspect when insect meal is equated as an ingredient for aquafeeds is its nutritional quality. Insect composition, and therefore its nutritional quality, is dependent of several factors, such as life stage, rearing conditions of insects and characteristics of the diet utilised for them. In general, insect meal is abundant in protein and lipids, but also contains chitin that may unpaired its digestibility when utilised as feed.

One of the most important criteria to be considered for feed protein sources is the percentage and quality of protein, which is characterized by its balanced EAA profile. Depending on the fish species, the protein requirement varies between 28 to 55% of dry diets. Considering that, high quality FM contains up to 73% of protein and soybean meal around 50% protein, insect meal which protein content range from 50 to 82% (dry matter) seems to be a good alternative protein source. Also in terms of AA profiles most of insect meals used in fish diets seems to be consistent with the fish requirements values. The EAA profile of insect meal seems to be more dependent on the taxonomic group of the insect rather than on the insect diet. Comparing the essential and limiting AA of FM and insect meal, the insect meal from the Diptera group has the more favorable EAA profile for aquafeed (Fig. 3) (Barroso *et al.,* 2014; Sànchez-Muros *et al.,* 2014; Henry *et al.,* 2015).

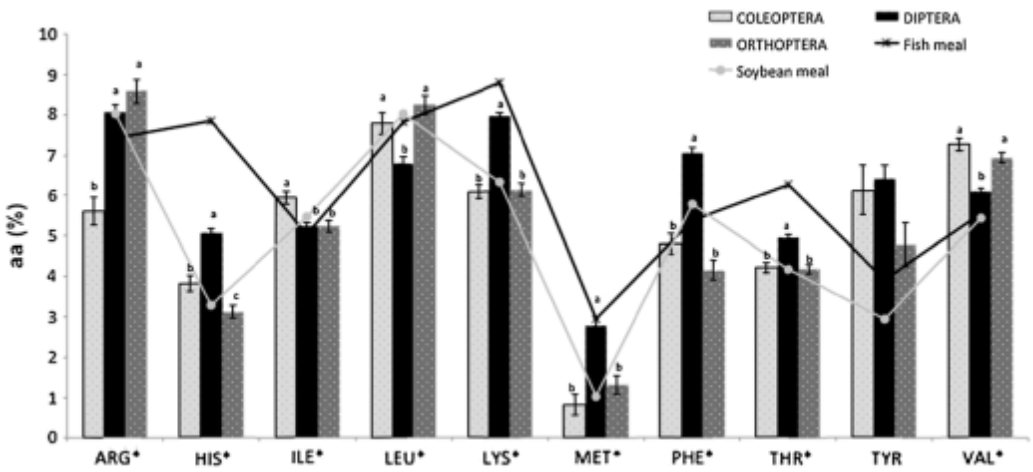


Figure 3. Percentage of essential amino acids in different insect orders, using FM and soybean meal as reference (Barroso *et al*., 2014).

Insects composition in terms of lipids and FAs depends on the species, development stages and the diet that insects are feed. Insect meal lipid level varies between 10 to 30%, being higher than that of FM (8.2%) and soya meal (3.0%). The quality and quantity of lipids can be modified during culture (Sánchez-Muros *et al.*, 2014).

Freshwater species can actively synthesize de novo LC-PUFA, of both n-3 and n-6 series, if their diet includes C18 PUFA precursors. However, marine fish species do not possess the enzymes necessary for the desaturation and elongation steps to produce these LC-PUFA of n-3 series, EPA and DHA, and so these FAs must be supplied by the diet. Unlike FM, which possesses high levels of EPA and DHA, terrestrial insects are deficient in these two essential FAs. Still aquatic insects are normally richer in EPA than terrestrial insects, since they feed on aquatic organisms that possess many n-3 FAs. For this reason, using aquatic insects as a source for insect meal seems a viable choice to compensate the lack of EPA (Sànchez-Muros *et al.,* 2014; Henry *et al.,* 2015).

Chitin is a carbohydrate polymer, that is present in invertebrate exoskeletons. Its composition differs between species and development stages *(*Belluco *et al.,* 2013*;* Sànchez-Muros *et al.,* 2014). It is assumed that fish cannot digest chitin, although studies show that the three enzymes (chitinase, chitobiase and lysozyme) responsible for chitin digestion were found in carnivorous and omnivorous fish, making this a subject of debate (Henry *et al.,* 2015). Even though it is suggested that soft bodied insects possessed less chitin and thus they being easier to digest than hard bodied insects. This may depend on the insect species, as it was shown in studies that larvae and adult crickets have similar chitin content (Henry *et al.,* 2015). In spite of reports

regarding the activity of chitinase in many fish species and benefits of chitin in marine fish, the presence of chitin continues to limit the use of insects in aquafeeds (Kroeckel *et al.,* 2012; Tran and Makkar*.,* 2015).

At present, a complete replacement of FM with insect meal has not been fully achieved, probably due to dietary unbalance or deficiencies. However, trials in which fish were feed with whole or cut insect larvae as protein source showed identical results with control fish feed low quality commercial diets (Henry *et al.,* 2015).

Also for European sea bass, the potential of mealworm meal of *Tenebrio molitor* as feed ingredients was study. The inclusion at 25% of mealworm meal in diets had no adverse outcome concerning weight gain, but modulated the FA composition of body lipids (Tran and Makkar*.,* 2015), whilst higher replacement levels (up to 50%) showed negative effects on growth.

Nevertheless, there is an urgent need for more studies to determine the nutritive characteristics of these insects, discuss potential constrains and find ways to improve the quality and acceptability of insect meal for fish as well as to evaluated the possible modulation effect of insect meal on fish quality. Certainly, utilization of insects in animal feeding seems to have a great potential in the future (Barroso *et al.,* 2014).

5. Black soldier fly

The black soldier fly (BSF) (*Hermetia illucens*) is a fly of the Diptera order and the Stratiomyidae family and is distributed around the tropics and warm temperate areas. Commonly known as latrine larvae, this species demands a warm environment resulting in a life cycle that varies between several weeks to several months relying on temperature and food conditions. *H. illucens* biggest advantages, besides being an extremely resistant species, are the possibility to be easily mass produced and the fact that adults are not a disease vector and do not need to be fed, since they depend only on the fat stored during larval stage (Makkar *et al.,* 2014).

It is a fact that many environmental problems concerning manure and other organic waste can have a solution by farming BSF larvae. This species can be feed on organic waste of low value, transforming it in biomass rich in protein, thus turning what is considered a waste into profitable biomass. Therefore, the conversion of organic wastes into protein-rich and fat-rich biomass can be a useful innovative alternative for many industries, contributing for the circular economy (Kroeckel *et al.,* 2012; Makkar *et al.,* 2014).

According to some studies about the different groups of insects, Diptera is the most similar to FM in terms of AA composition, especially the larvae of *Hermetia illucens* (Barroso *et al.,* 2014). *H. illucens* larvae are considered an important feed source since they are rich in protein and fat. Although they possess around 40-44% of crude protein, the amount of fat and FA composition strictly depends on the type of diet they are fed. The presence of chitin in BSF pre-pupae might bring consequences to digestibility and nutrient utilization (Kroeckel *et al.,* 2012; Makkar *et al.,* 2014). Research on the dietary inclusion of black soldiers prepupae meal revealed a great growth of some fish species, such as rainbow trout *(Oncorhynchus mykiss)*, channel catfish *(Ictalurus punctatus)* and blue tilapia *(Oreochromis aureus)* (Bondari and Sheppard, 1981; St-Hilaire *et al.,* 2007a; Stamer *et al.,* 2008; Van Huis *et al.,* 2013). The effect of black soldiers prepupae meal, particularly of its FA profile, on rainbow trout flavor and aroma was studied and showed that muscle FA profile was sensibly affected (Sealey *et al*., 2011). Rainbow trout fed diets with BSF meal and lower levels of fish oil led to a reduction of n-3 FA levels in fillet muscle (St-Hilaire *et al.,* 2007a). For this reason, there are some concerns about the alteration on taste, aroma and quality of fish fed diets with BSF (Riddick, 2014). Sensory analysis studies on rainbow trout, tilapia and channel catfish revealed no significant differences between fish fed the control diet containing FM as compared with fish fed diets with BSF (Bondari and Sheppard, 1981; Sealey *et al*., 2011).

In general, many studies have shown that FM can be adequately replaced by BSF larvae meal, either partially or totally (Bondari and Sheppard, 1981; Newton *et al*., 2004; St-Hilaire *et al.,* 2007a; Sealey *et al*., 2011).

6. Fish Quality Parameters

In order to introduce a new ingredient on fish diets there is the need to unsure that this change will not modify fish quality parameters, so they can safely enter the food market (Wu and Sun, 2013). Moreover, the growing attention on the numerous benefits of fish consumption led to an increasing concern about safety and quality of fish products. Fish is an important part of our diet consumed by human, and it follows a strict regulation in terms of food safety. The existence of an effective control mechanism normally withdraws potentially harmful products before they are commercialized, so only safe products reach the consumers (FAO, 2014).

Visual appearance is a very important property in the food industry. Quality parameters such as chemical composition, colour and texture, among others, are essential in

evaluating fish flesh quality. These parameters are influenced by various factors, for instance fish species, size, sexual maturity, season, temperature and source of nutrients *(*Fuentes et al., 2010). Quality parameters are important criteria that affect the purchasing of a food product, as it is usually used to assess the quality of the product. Fish visual appearance is greatly affected by skin and muscle colour as consumers tend to associated freshness, flavor and quality with the colour of the fish (Wu and Sun, 2013). Another important property determining the fish quality is its texture, that is a very complex parameter described by its juiciness, chewiness and dryness and that normally is evaluated together with flavor characteristics. Texture attributes of fish fillet can be altered by its lipid content. Studies by Andersen *et al.* (1997) revealed that fish fed a diet with high lipid levels resulted in softer fillets than fish fed low lipid diet. Many questions are still unanswered about the consequences of fish nutrition on fish quality (Huss, 1995; Lie, 2001).

Lipids have an important role in quality parameters since they affect the sensory attributes of food, such as colour, texture, aroma and flavor (Sikorski and Kalakowska, 2002). For that reason, fillet lipids, in terms of quantity, quality and FA profile, are often used to assess the quality parameters. Overall, wild fish are leaner than farmed fish, depending on the species of fish and on the quality and type of feed utilised during rearing process. Aquafeed is known to modulate the flesh composition, especially the lipid content and FA profile of feed and information related to these characteristics can be utilized to manipulate carcass and fillet quality of fish, considering the preferences of consumers. Despite the information that dietary lipid level is responsible for the carcass lipid deposition, there still are doubts about the optimal dietary lipid level and its FA profile to obtain the optimum fillet quality for each fish species. Moreover, in species with high whole-body lipid level, it was shown that lipids in the fillet are not uniformly distributed in it (Lie, 2001; Fuentes *et al.,* 2010).

Fish food quality is greatly influenced by the chemical modifications of lipids that occur during storage and processing, among other changes that characterize the raw material. One of the deleterious consequences of lipids in the muscle is lipid oxidation. In fact, in food storage at refrigerated or frozen temperatures one of the principal reasons for quality deterioration in the muscle is represented by this complex chemical process. Food oxidation depends on three factors: the FA profile in the food matrix, the pro and antioxidant status and the external elements as light, oxygen pressure, and temperature. Since lipids present in fish carcass are rich in PUFA, this makes fish lipids highly susceptible to peroxidation. This type of oxidation deteriorates nutritive value and results in sensory changes of the product that will influence food quality and

consumption (Sikorski and Kalakowska, 2002; Azhar, 2006) but also the safety characteristics.

Lipid oxidation results in lipid hydroperoxides as a primary oxidation product. However, the quantification of these products is not considered an index of quality, since hydroperoxides do not affect flavour or odour and so they are not connected to the sensory quality of the product, but may suggest the formation of products that will affect sensory quality. The secondary oxidation products involve aldehydes, ketones, short chain FA among others, that are characterized by unpleasant odour and flavour that give the fish rancid flavour. The determination of the secondary products is based on the knowledge that there is a formation of a reddish coloured product from the reaction between aldehydic products and thiobarbituric acid. The reddish coloured products can be determined spectrophotometrically thus obtaining a measure of thiobarbituric acid-reactive substances (TBARS) (Huss, 1995).

The composition of aquaculture fish can be controlled, to some degree, by the composition of feed. Manipulating the ingredient and nutrient composition of fish diets can modulate the quality and safety, increasing or compromising the value of the final products and also deliver health benefits or constrains to the consumers. Therefore, it is of upmost importance to assess the effect of the dietary incorporation of innovative alternative protein sources on seafood quality, to ensure the maintenance of the best quality of the final product (Oliva Teles, 2000; FAO, 2014). This is the case of the insect meal as innovative ingredient for aquafeed.

Objectives

The search for new alternative ingredients to FM, the premium protein sources in aquafeeds, is increased due to the continuous worldwide development and industrialization of aquaculture. Nonetheless, for the correct use of these new ingredients, it is of major importance to have a holistic approach of the effects of its dietary inclusion, not only on fish growth performance and feed utilization, but also on fish quality parameters, to ensure the safety, high quality and the human health benefits of eating seafood.

Insect meal has been pointed out to have high potential as a sustainable protein source alternative to FM. The development of mass production of insects together with the increased price of FM and traditional plant protein sources, provide interesting perspectives for the use of insects in aquafeeds. Moreover, insect production

maximizes the benefits of waste management by using ‘‘waste” by-products for insect growth and does not compete with food resources or land use. Additionally, the nutritional profile of insect meal, in terms of protein, lipids and EAA and FA profiles make it potentially suitable to be incorporate in aquafeeds.

Due to the importance of fish quality for human consumption, it is of utmost importance to study the possible interaction between insect meal based diets and fish quality traits that can influence consumer’s choice. In this context, the current study was conducted to evaluate the effect of dietary FM replacement by insect meal on quality attributes of fish as well as freshness evolution and changes in the quality parameters during shelf life of European sea bass (*Dicentrarchus labrax*).

Material and Methods

This study was developed in collaboration with the NUTRIMU group from CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto (Portugal) and the Department of Agri-Food Production and Environmental Sciences (DISPAA), Agriculture School, University of Florence (Italy).

In this experiment, the formulation of the experimental diets, the growth trial and tissue sampling was performed in the Marine Zoological Station of the Faculty of Science in the University of Porto. This growth trial was performed by another student as a part of his master thesis work. At the end of the growth trial, some of these fish were utilised to evaluate fillet quality, fillet sensory profile and fillet texture of both freshly caught and throughout the shelf life period. These analyses were performed in DISPAA at the University of Florence, that received the samples from Portugal.

1. Formulation of the Experimental Diets

Four experimental diets were formulated to be isoproteic and isolipidic, according Peres and Oliva-Teles (1999, 2002). Control diet was formulated including 32% of FM and no insect meal, represented by BSF (*Hermetia illucens*) prepupae meal. In the test diets, increased levels of insect meal were incorporated replacing FM at 6.5, 13 and 19.5% dry matter, which corresponded to a protein replacement level of 7.5, 15, 22.5% of the total dietary protein. All dietary ingredients were finely ground, well mixed and dry pelleted in a laboratory pellet mill (CPM) through 3 mm dye. After drying, the pellets were moved in a drying oven at 55 ºC for 24 hours, and the feeds obtained were stored at -20 ºC until use.

The ingredient composition and the proximate analysis of the diets are shown in Table 1. Amino acid and fatty acid profile of insect meal and the experimental diets are presented in Table 2 and Table 3, respectively.

Table 1. Ingredient composition, proximate analysis and gross energy content of the experimental diets.

	HM0	HM6.5	HM13	HM22.5
Ingredients (g 100 g^{-1})				
Fish meal[1]	32.4	27.5	22.7	17.8
Insect meal[2]	0	6.5	13	19.5
Corn gluten[3]	10	10	10	10
Soybean meal[4]	12	12	12	12
Wheat gluten[5]	7.1	7.4	7.6	7.9
Wheat meal[6]	17.9	15.7	13.6	11.4
Fish oil[7]	12.9	13.2	13.5	13.7
Vitamin premix[8]	1	1	1	1
Choline chloride (50%)	0.5	0.5	0.5	0.5
Mineral premix[9]	1	1	1	1
Binder[10]	1	1	1	1
Agar	1	1	1	1
Chromic oxide (Cr_2O_3)	0.5	0.5	0.5	0.5
Taurine	0.2	0.2	0.2	0.2
NaH_2PO_4	0	0.4	0.8	1.2
Cellulose	2.5	2.1	1.7	1.3
Proximate analysis				
Dry matter (%)	91.5	93.3	94	92.2
Crude protein (% dry weight)	48.1	48.9	50.4	52.8
Crude lipid (% dry weight)	17.5	17.1	17.4	17.9
Ash (% dry weight)	9.2	10.5	9.6	9.5
Grosse energy (kJ g^{-1})	22.8	23.1	23.1	23.1

[1] Fish meal, Pesquera Centinela, Steam Dried LT, Chile (CP: 69.6%; CL 11.3%). Sorgal, S.A. Ovar, Portugal.
[2] Insect meal (CP: 55.8%; CL: 5.5%; ash: 8.5%), Hermetia Deutchland GmvH & Co KG. Baruth/Mark, Germany.
[3] Corn gluten (CP: 78.6%; CL: 4.1%), Sorgal, S.A. Ovar, Portugal.
[4] Soybean meal (CP: 55.1%; CL: 2.5%), Sorgal, S.A. Ovar, Portugal.
[5] Wheat gluten (CP: 85.6%; CL: 1.74%), Sorgal, S.A. Ovar, Portugal.
[6] Wheat meal (CP: 9.9%; CL: 3.2%), Sorgal, S.A. Ovar, Portugal.
[7] Fish oil, Sorgal, S.A. Ovar, Portugal.
[8] Vitamins (mg kg^{-1} diet): retinol, 18000 (IU kg^{-1} diet); calciferol, 2000 (IU kg^{-1} diet); alpha tocopherol, 35; menadion sodium bis., 10; thiamin, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400.
[9] Minerals (mg kg^{-1} diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dicalcium phosphate, 8.02 (g kg^{-1} diet); potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.4 (g kg^{-1} diet).
[10] Aquacube. Agil, UK.

Table 2. Fatty acid profile of insect meal and experimental diets (% total fatty acid).

Fatty Acid	Insect Meal	HM0	HM7.5	HM15	HM22.5
C12:0	43.53	0.47	1.01	1.96	2.67
C14:0	8.29	3.73	3.88	3.94	3.98
C16:0	14.78	15.13	15.49	15.46	15.57
C18:0	2.73	3.74	3.71	3.69	3.71
C16:1n-7	4.2	4.66	4.71	4.63	4.56
C18:1n-9	13.32	14.68	15.07	15.19	15.36
C18:1n-7	0.39	2.17	2.15	2.09	2.03
C20:1n-9	0.14	2.15	2.14	2.12	2.08
C18:2n-6	8.69	8.41	8.61	8.73	8.86
C20:4n-6	0.05	1.5	1.45	1.4	1.35
C18:3n-3	1.17	1.43	1.46	1.47	1.48
C18:4n-3	0.04	1.46	1.42	1.39	1.36
C20:5n-3 (EPA)	0.21	9.62	9.13	8.75	8.36
C22:5n-3	0.33	2.76	2.34	2.23	2.23
C22:6n-3(DHA)	0.46	18.86	18.18	17.99	17.54
Σ SFA	69.92	24.61	25.66	26.63	27.49
Σ MUFA	18.84	27.54	28.01	27.84	27.87
Σ PUFA	11.24	47.86	46.33	45.53	44.64
Σ n-6	8.83	11.13	11.26	11.32	11.39
Σ n-3	2.34	35.25	33.66	32.9	32.01

The fatty acids C13:0, C15:0, C17:0, C20:0, C22:0, C24:0, C14:1n-5, C16:1n-9, C20:1n-11, C20:1n-7, C22:1n-11, C22:1n-9, C22:1n-2, C18:3n-6, C20:2n-6, C20:3n-6, C22:2n-6, C22:4n-6, C22:5n-6, C20:3n-3, C20:4n-3, C21:5n-3, C16:2n-4, C16:3n-4, C18:2n-4, C18:3n-4, C16:4n-1, C18:4n-1, in percentage <1% were also detected but not reported in the table for brevity. They were utilized to calculate the fatty acid groups.

Table 3. Amino acid composition of insect meal and experimental diets (g 16 g^{-1} N).

	Insect meal	HM0	HM7.5	HM15	HM22.5
Essential amino acids					
Isoleucine	7.64	10.05	10.64	10.21	9.79
Leucine	4.10	7.98	9.31	8.12	7.21
Lysine	4.26	8.12	5.63	4.28	4.38
Methionine	1.62	2.30	2.18	2.03	1.89
Phenylalanine	4.19	5.74	4.41	4.83	5.76
Threonine	6.10	3.41	3.08	3.75	3.86
Valine	6.38	4.79	5.38	4.69	4.86
Arginine	4.84	5.24	5.43	4.80	4.82
Histidine	2.83	2.08	1.49	2.53	2.39
Non-essential amino acids					
Alanine	8.09	5.52	5.73	6.10	6.47
Aspartate	9.84	7.14	6.32	7.79	7.99
Cysteine	2.58	2.39	2.73	2.31	2.34
Glutamate	12.25	16.82	18.12	18.32	18.1
Glycine	5.29	4.21	4.45	4.30	4.37

Proline	6.24	5.79	6.61	6.45	6.04
Serine	7.49	4.50	4.82	5.21	5.41
Tyrosine	6.10	3.41	3.08	3.75	3.86
Taurine	0.13	0.50	0.59	0.53	0.47

2. Feeding Trial

The growth trial was conducted at the Marine Zoological Station, Faculty of Science, University of Porto (Portugal), with the European sea bass (*Dicentrarchus labrax*) juveniles provided by MARESA. Mariscos de Estero S.A. Company (Finca El Tambujal s/n, Apdo de correo 82. Ayamonte, Huelva, Portugal). The experimental system consisted in a thermoregulated recirculation water system, equipped with twelve fiberglass tanks of 60 liters water capacity. During the trial, temperature was maintained constant at 26 ºC, salinity averaged 36 g l^{-1} and dissolved oxygen was kept above 80% of saturation. The experimental fish were subjected to a 12 h light/12 h dark artificial photoperiod regime.

At the beginning of the trial, 12 groups of 10 European sea bass juveniles were used (initial body weight of 49.4±0.5 g) and were established in each tank. Each experimental diet was randomly assigned to triplicate groups of fish, that were hand-fed until visual satiation, twice a day, 6 days a week, over a period of 62 days.

3. Tissue sampling

At the end of the growth trial, 3 fish per tank were randomly sampled from each group, 4 hours after the morning meal. Muscle samples were also taken from the lateral dorsal part of the body, below the dorsal fin and above the lateral line, and frozen at -80 ºC, for study of quality traits and quality changes, during shelf life, at two different times, T0 that corresponded to the initial time and T3 that corresponded to three days of fillets storage in a fridge at a temperature of +2/4 ºC. Another 3 fish from each tank were sampled as whole fish for wet weight, length, physical and chemical analyses

4. Physical Analyses

After slaughtering, the fish and fillets were transported in dry ice to the laboratory of DISSPA, Florence (Italy) and were then kept at a -80 ºC to avoid any deterioration process to occur.

After a very short period of storage, the fish were dissected, and the head, total cavity content, liver, perivisceral fat, and right and left fillet were weighed. Liver physical

characteristics, including colour, were measured. Then liver was homogenized for evaluation of total lipid content. Both fillets were also collected, and their colour was measured; after the fillets were skinned and homogenized, and proximate analyses were carried out on samples taken from the fillet muscle.

Fillets samples of fish previously dissected at the Marine Zoological Station of the University of Porto (Portugal) were analyzed at two different *post mortem* times, T0 and T3, being T0 the initial time and T3 three days of storage in a fridge at a temperature of + 2/4ºC. Time was decided considering the period of commercial life that is the most common in the fish trade as currently organized in Italy by the large scale distribution retail market. Physical characteristics, such as colour and pH, were analyzed directly on the left and right fillet at T0. The left fillet was skinned, homogenized and stored to perform chemical analysis of lipid oxidation products and total lipid content at T0. The right fillets were stored on a fridge at +2/4 ºC temperature without ice for three days, after which physical analyses were performed again, this being T3. The right fillet was then skinned, homogenized and samples were taken to perform chemical analysis of lipid oxidation products and total lipid content at T3. All samples taken for lipid oxidation products and total lipid content were frozen at -80 ºC until further analysis.

4.1 Colour

A Spectro-colour colorimeter (using Spectral qc 3.6 software) was utilized for colourimetric measurements carried out according to the CIE Lab system (CIE, 1976). In this system, lightness (L*) is expressed on a 0-100% scale from black to white, redness index (a*) ranges from red (+60) to green (-60) while yellowness index (b*) ranges from yellow (+60) to blue (-60).). In addition, the values of Chroma, as a measure of colour saturation, and Hue as perception of colour were also recorded. Colour was measured in triplicate on the epaxial fillet position. In the liver colour was measured only in one position due to its small size. (**Fig. 4**).

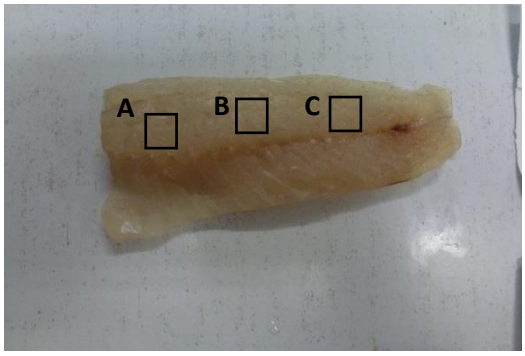


Figure 1. Fillet of European sea bass showing the three positions where the colour was measured. Photo: Rita Pedrosa.

4.2 pH

The pH of the muscle at T0 and T3 was measured in three different epaxial fillet positions, with a Mettler Toledo (Novate Milanese, Milano, Italy), Mod. DevenGo SG2, pHmeter.

5. Total Lipid Content

Total lipid quantification of muscle at T0and at T3 and of liver was performed according to Folch et al. (1956). Briefly, total lipid content was determined gravimetrically after removal of the solvent (chloroform) by evaporation under vacuum and lipid resuspension in a known volume of chloroform (5 ml). Then, 0.5 ml of lipid extract was weighed in a crucible and, after complete evaporation of chloroform in an oven at 105 °C for 5 minutes, lipid content was gravimetrically determined. The extracted lipids were utilised for the FA profile analysis and for measuring the lipid oxidation products.

6. Fatty Acid Analysis

Fatty acid methyl esters (FAME) composition of muscle at T0 and at T3 and of liver was analysed according the modified method of Morrison and Smith (1964), as summarized in Francesco *et al.* (2004). Briefly, lipids were saponified with 0.5 M KOH in methanol, and FAs were hydrolysed by adding 2.5 ml of 2 M HCl. Methyl esters were prepared by transmethylation, using 2 ml boron fluoride-methanol at a 14% concentration. Methylated FAs were dissolved in petroleum ether, dried, and finally resuspended in 1.5 ml of hexane.

The FA composition was determined by liquid gas chromatography (LGC) using a Varian GC 430 gas chromatograph (Varian Inc., Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a Supelco Omegawax™ 320 capillary film (30 m x 0.32 mm i.d., 0.25 µm) and polyethylene glycol bonded phase (Supelco, Bellefonte, PA, USA). The oven temperature was held at 100 °C for 2 minutes, increased to 160 °C over 4 minutes at a rate of 12 °C/min, after that the oven was increase to 220 °C over 14 minutes at the rate of 3 °C/min, and kept at 220 °C for 25 minutes. The injector and the detector temperatures were set at 220 °C and 300 °C, respectively. One microlitre of sample in hexane was injected into the column with the carrier gas (helium) at a constant flow of 1.5 ml/min. The split ratio was 1:20. Chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 (Agilent) computing integrator software. FAs were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix Supelco (Bellefonte, PA,

USA). FAs were quantified through calibration curves, using tricosanoic acid (C23:0) (Supelco, Bellefonte, PA, USA) as an internal standard.

7. Fat Quality Indexes

The FA composition was utilised to determine quality indexes of lipid profile, with the intention to evaluate nutritional and health aspects of sea bass fillets. The atherogenicity index (AI) and thrombogenicity index (TI) were calculated according to Ulbricht and Southgate (1991), while the hypocholesterolemic to hypercholesterolemic FAs ratio (h/ H) according to Santos-Silva et al. (2002), using the following formulae:

Atherogenicity index (AI):

[C12:0 + (4 × C14:0) + C16:0] /(Σ PUFAn-3 + Σ PUFAn-6 + Σ MUFA);

Thrombogenicity index (TI):

[C14:0 + C16:0 + C18:0]/[0.5 × Σ MUFA) + (0.5 × Σ PUFAn-6) + (3 ×Σ PUFAn-3) + (Σ PUFAn-3/ Σ PUFAn-6)];

Hypocholesterolaemic/hypercholesterolaemic FA ratio (HH):

(C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3+ C22:5n-3 + C22:6n-3) / (C14:0 + C16:0) .

Furthermore, PUFAn-3/PUFAn-6 and PUFA/SFA ratios were also calculated. To calculate the quality indexes, the FAs concentrations were expressed as g /100 g of fillet.

8. Lipid Oxidation Products

The lipid oxidation products were determined in the muscle at T0 and T3.

The content of conjugated dienes (CD) in the lipid extract was measured by a colourimetric method, using hexane as solvent. CDs were quantified at 233 nm (50 Scan spectrophotometer equipped with Cary Win UV software, Varian, Palo Alto, CA, USA), using a molar extinction coefficient of 25 200 l mol^{-1} cm^{-1}. Results were expressed as mol CD kg^{-1} sample.

The content in 2-thiobarbituric acid-reactive substances (TBARS) was measured by a colourimetric method at 532 nm. Briefly, TBARS were extracted in 50 g l^{-1} Trichloroacetic acid (TCA) and added with 0.04 mol l^{-1} tertiary butyl alcohol (TBA). After 20 min of incubation at 93 °C, the products were quantified with reference to a calibration curve of 1.1.3.3-tetraethoxypropane (TEP) in 50 g l^{-1} TCA (0.8–8 µmol l^{-1}).

9. Proximate analysis

Proximate analysis of the insect meal, experimental diets and fillets were conducted following the AOAC procedures (AOAC, 2000) 950.46, 976.05, 991.36, and 920.153 methods, for protein (N x 6.25), lipid, and ash content, respectively.

Dry matter was determined after drying at 105 °C until constant weight; ash by incineration in a muffle furnace at 450 °C for 16 hours; crude protein content (N × 6.25) by the Kjeldahl method after acid digestion using a Kjeltec digestor and distillation units (Tecator Systems, Höganäs, Sweden; models 1015 and 1026, respectively), lipids by petroleum ether extraction in a SoxTec extraction system (Tecator Systems; extraction unit model 1043 and service unit model 1046) and gross energy by direct combustion in an adiabatic calorimeter bomb (PARR Instruments, Moline, IL, USA; PARR model 1261).

10. Statistical Analysis

Data were analysed by one-way analysis of variance (ANOVA) and two way ANOVA. Normality and homogeneity of variance were checked by the Shapiro–Wilk test and Levene's test, respectively, and transformed if necessary. The probability level of 0.05 was used for rejection of the null hypothesis. Significant differences among groups were determined by the Tukey's multiple range test. Statistical analysis was performed using SPSS 20.0 software package as the statistical software.

Results

FA profile, expressed as percentage of total FA, of experimental diets and insect meal is presented in **Table 2**. In insect meal, saturated fatty acid (SFA) accounted to 69.9% of the total FA, being the C12:0 and C16:0 the most abundant fatty acids. Among the LC-PUFA, the n-3 FAs were less abundant than the n-6, totalizing 2.3 and 8.8% of total FA, respectively. In the experimental diet, the increase of FM replacement level by insect meal resulted in an increase of SFA, from 24.6 to 27.5% of total FAs, in control diet and HM22.5, respectively, while n-3 LC-PUFA were reduced from 35.2 to 32.0% of total FA, with EPA and DHA being the most abundant FAs.

Amino acid composition of insect meal and the experimental diets is shown in **Table 3**. In insect meal, the most abundant essential amino acid was isoleucine and the less abundant methionine. Thus, the increase of insect meal replacement level in diets resulted in a decrease of lysine and methionine content.

At the end of the feeding trial, the dietary FM replacement by increased level of insect meal did not affect growth performance or feed utilization (data not shown). Similarly, morphometric parameters as well as fillet yield, viscerosomatic, hepatosomatic and mesenteric fat indices were also not affected (**Table 4**). The fillet proximate composition was also unaffected by the dietary treatment, except for the ash content that was higher in fish fed the control diet relatively to HM22.5 and HM7.5 diets (**Table 5**).

Table 4. Body morphological traits of European sea bass fed the experimental diets.

	HM0	HM7.5	HM15	HM22.5	SEM
Morphometric data					
Body weight (g)	112.6	135.4	126.2	116.6	4.1
Total length (cm)	20.5	21.4	20.6	20.4	0.2
Fillet yield[1] (%)	47.0	47.6	47.3	45.7	0.5
Viscerosomatic index[2] (%)	10.7	12.3	12.1	11.9	0.3
Hepatosomatic index[3] (%)	1.1	1.1	1.4	1.2	0.1
Mesenteric fat index[4] (%)	3.5	4.4	3.8	4.2	0.2

SEM: pooled standard error of the mean.
[1] Fillet yield (%): (fillets with skin weight / body weight) × 100
[2] Viscerosomatic index (%): (viscera weight / body weight) × 100
[3] Hepatosomatic index (%): (liver weight / body weight) × 100
[4] Mesenteric fat index (%): (mesenteric fat weight / body weight) × 100.

Table 5. Fillet composition of European sea bass fed the experimental diets.

Diets	HM0	HM7.5	HM15	HM22.5	SEM
Dry matter (%)	6.9	7.0	6.8	6.5	0.1
Crude protein (% wet weight)	78.3	77.3	78.9	76.6	0.8
Crude fat (% wet weight)	9.6	11.0	9.4	12.1	0.9
Ash (% wet weight)	5.3^{b}	4.8^{a}	5.1^{ab}	4.8^{a}	0.1

SEM: pooled standard error of the mean.

Total liver lipids, monounsaturated (MUFA), n-3 and n-6 PUFA fatty acids and the n-3/n-6 ratio were not changed with the diet (**Table 6)**

Table 6. Total lipids and fatty acid profile (% total fatty acids) in liver of European sea bass juveniles fed the experimental diets.

	HM0	HM7.5	HM15	HM22.5	SEM
Total lipids **(% as is)**	24.31	22.97	24.36	24.33	0.72
Fatty acids					
C14:0	2.14	2.03	2.09	2.08	0.03
C16:0	19.44	19.77	19.44	18.97	0.41
C18:0	5.51	5.55	5.1	4.84	0.2
C16:1n-7	3.85	3.85	3.8	3.96	0.05
C18:1n-9	30.25	32.91	30.54	30.92	0.88
C18:1n-7	2.37	2.46	2.44	2.48	0.03
C20:1n-9	1.5	1.47	1.6	1.49	0.02
C18:2n-6	5.04	4.79	5.34	5.31	0.2
C20:4n-6	1.18	1.18	1.23	1.18	0.04
C20:5n-3(EPA)	4.69	4.64	5.11	4.64	0.15
C22:5n-3	1.59	1.47	1.62	1.65	0.08
C22:6n-3(DHA)	13.71	13.51	14.91	15.36	0.7
Σ SFA	28.16	28.54	27.95	26.85	0.55
Σ MUFA	41.8	43.11	40.98	41.36	0.77
Σ PUFA	29.32	28.62	31.73	31.77	0.81
Σ n-6	7.38	7.12	7.75	7.71	0.26
Σ n-3	21.47	21.08	23.47	23.59	0.99
Ratios					
n-3/ n-6	3.02	3.08	3	3.04	0.05
PUFA/SFA	0.98	1	1.1	1.25	0.07

The fatty acids C12:0, C13:0, C15.0, C17:0, C20:0, C22:0, C24:0, C14:1n-5, C16:1n-9, C20:1n-11, C20:1n-7, C22:1n-9, C22:1n-7, C22:1n-11, C18:3n-6, C20:2n-6, C20:3n-6, C22:2n-6, C22:4n-6, C22:5n-6, C18:3n-3, C18:4n-3, C20:3n-3, C20:4n-3, C21:5n-3, C16:2n-4, C16:3n-4, C18:2n-4, C18:3n-4, C16:4n11, C18:4n-1, in percentage <1%, were also detected but not reported in the table for brevity. They were utilized to calculate the fatty acid groups. SEM: pooled standard error of the mean.

Colour measurements of fish skin, fillet muscle and liver were not affect by the dietary treatment (**Table 7**). The only expectation was the colour saturation (Chroma) on the epaxial right fillet which was higher in fish fed the diet HM15 than in those fed the HM7.5 diet, but a clear trend of this parameter with the increasing level of insect meal in the diet was not highlighted.

Table 7. Colour parameters (L*, a*, b*, Chroma and Hue) skin, right fillet, left fillet in the liver,, epaxial and hypaxial regions of European sea bass juveniles fed the experimental diets.

	Diets				SEM
	HM0	**HM7.5**	**HM15**	**HM22.5**	
Skin					
Dorsal					
L*	44.26	41.16	44.18	45.98	1.37
a*	-1.20	-1.63	-1.59	-1.57	0.07
b*	-0.39	-0.58	0.46	-1.11	0.35
Chroma	3.09	3.04	2.74	2.48	0.26
Hue	180.45	191.77	174.25	193.80	7.76
Ventral					
L*	73.14	75.49	74.79	74.83	0.61
a*	-1.95	-2.05	-1.93	-1.93	0.05
b*	2.62	1.30	2.37	2.05	0.38
Chroma	4.03	3.33	3.71	3.00	0.20
Hue	147.65	138.25	148.13	138.76	4.78
Right fillet					
Dorsal					
L*	29.04	29.26	28.44	29.66	0.63
a*	-2.16	-2.84	-2.17	-1.64	0.18
b*	5.98	4.17	6.64	5.28	0.38
Chroma	6.69^{ab}	5.03^{a}	7.29^{b}	5.85^{ab}	0.33
Hue	110.66	125.17	112.18	111.27	2.94
Ventral					
L*	38.17	36.23	38.19	37.21	0.94
a*	-0.67	0.21	0.31	-0.25	0.25
b*	11.54	10.77	11.78	11.24	0.32
Chroma	11.73	11.01	12.39	11.32	0.32
Hue	95.05	90.95	87.35	92.40	1.36
Left fillet					
Dorsal					
L*	30.58	28.30	28.80	31.03	0.61
a*	-2.04	-1.89	-1.23	-0.21	0.29
B*	5.88	4.82	7.23	6.93	0.36
Chroma	6.75	5.65	7.59	6.41	0.30
Hue	107.12	112.86	104.04	101.56	2.83
Ventral					
L*	42.44	39.27	44.07	41.76	0.97

a*	-1.00	-1.29	-0.92	-1.30	0.16
b*	10.41	9.26	10.71	10.18	0.33
Chroma	10.63	9.43	10.86	10.42	0.32
Hue	97.83	99.56	96.05	93.62	1.06
Liver					
L*	40.55	37.16	41.44	46.36	1.85
a*	14.69	15.48	12.75	9.86	1.27
b*	29.25	30.63	26.26	28.21	1.36
Chroma	33.07	39.45	29.48	29.91	1.76
Hue	65.02	60.30	65.45	70.93	1.68

SEM: pooled standard error of the mean. Means in the same row with different superscript letters are significantly different ($p < 0.05$).
L*: lightness
a*: redness index
b*: yellowness index.

The effect of storage time at +2/4 ºC on pH and colour parameters (L*, a*, b*, Chroma and Hue) of fish fed the experimental diets are reported in **Table 8**. Neither storage time nor experimental diets affected fillet length, weight and colour parameters. Irrespectively of the storage time, fillet pH of fish fed the HM22.5 was higher than that of fish fed the control (HM0) diet.

The effect of storage time on total lipid content and primary and secondary oxidation products in fillets of fish fed the experimental diets are presented in **Table 9**. The total lipid content of fish fillets was not affect by either storage time or dietary treatment. Storage for 3 day at +2/4 ºC increased both CDs and TBARS levels in the fish fillets. Moreover, irrespectively the storage time, the CD level was higher in fish fed diet HM15 and HM22.5 than in those fed the HM0 and HM7.5 diets. At T3, TBARS levels were higher in control that in HM22.5 diet.

Table 8. Fillets, pH and colour parameters (L*, a*, b* Chroma, Hue) of European sea bass juveniles fed the experimental diets, before and after 3 days of storage.

Parameters	Storage Time (days)									
	0					3				
	HM0	HM7.5	HM15	HM22.5	SEM	HM0	HM7.5	HM15	HM22.5	SEM
pH	6.26	6.30	6.30	6.37	0.02	6.28	6.29	6.28	6.35	0.02
L*	45.67	43.84	44.06	44.63	0.03	49.16	46.13	48.79	44.91	0.07
a*	-1.54	-1.59	-1.45	-1.68	0.03	-1.61	-1.70	-1.56	-1.70	0.07
b*	3.87	3.76	4.05	3.62	0.03	5.02	4.41	4.05	4.66	0.07
Chroma	4.23	4.10	4.36	4.02	0.03	5.33	4.81	4.37	4.54	0.07
Hue	114.57	112.42	111.38	115.02	0.03	110.39	114.56	111.43	111.47	0.07

Two way ANOVA

	Variation Source			Diets			
	Diet	Time	Interaction	HM0	HM7.5	HM15	HM22.5
pH	*	NS	NS	a	ab	ab	b
L*	NS	NS	NS				
a*	NS	NS	NS				
b*	NS	NS	NS				
Chroma	NS	NS	NS				
Hue	NS	NS	NS				

Two-Way ANOVA: *p< 0.05; NS: non-significant. SEM: pooled standard error of the mean.

Table 9. Total lipids, primary (CDs) and secondary (TBARS) oxidation products of European sea bass fillets fed the experimental diets, before and after 3 days of storage.

Storage Time (days)	**0**					**3**				
Diets	**HM0**	**HM7.5**	**HM15**	**HM22.5**	**SEM**	**HM0**	**HM7.5**	**HM15**	**HM22.5**	**SEM**
Total lipids (g/100g)	4.18	4.30	5.03	5.32	0.21	4.35	5.03	5.87	5.39	0.29
CDs (mol kg^{-1} muscle)	0.22	0.22	0.26	0.29	0.01	0.25	0.24	0.34	0.34	0.01
TBARS (mg kg^{-1} muscle)	1.46	1.23	1.26	1.55	0.10	3.11	2.21	2.36	1.98	0.14

Two way ANOVA

	Variation source			**Diets**			
	Diet	**Time**	**Interaction**	**HM0**	**HM7.5**	**HM15**	**HM22.5**
Total lipids (g/100g)	NS	NS	NS				
CDs (mol kg^{-1} muscle)	**	***	NS	a	a	b	b
TBARS (mg kg^{-1} muscle)	*	***	NS	b	ab	ab	a

Two-Way ANOVA: *p< 0.05; ** p < 0.01; *** p < 0.001; NS: non-significant. SEM: pooled standard error of the mean.

The fillet FA profile of fish fed the experimental diets, before and after 3 days of storage at +2/4 ºC is presented in **Table 10**. Total n-3 PUFA, DHA (C22:6n-3) and C20:4n-6 FA were not affected by diets before storage, while after 3 days of storage it decreased with the increase of insect meal in diets. Total n-6 PUFA was not affected by storage time but it was higher in insect meal incorporating diets than in the control diet (HM0). The ratio n-3/n-6 and the C22:5n-3 FA percentage were decreased by the increase of the storage time. Before storage, C13:0 FA incidence was higher in the control fish than in HM15 diet fish; while not effect of dietary treatment was observed after 3 days of storage. Before storage, C22:1n-11 FA percentage was higher in HM0 group than in HM15 group, while after 3 days of storage it was lower in fish fed HM0 and HM7.5 than in HM22.5 fish. C16:1n-7 FA in fillets of fish fed the experimental diets HM0 and HM7.5 was higher than in fillets of fish fed HM15. The FAs C18:0 decreased in fish fed diets containing insect meal and was unaffected by storage time. Docosapentaenoic acid (DPA; 22:5n-3) only revealed significant differences between storage time, with decreasing values after 3 days of storage, irrespectively of the dietary treatment.

Lipid quality of fish fillets assessed by the indexes of atherogenicity, thrombogenicity and hypocholesterolemic/hypercholesterolemic FA ratio before and after 3 days of storage at +2/4 ºC were not affect by the dietary treatment or time storage (**Table 11**).

Table 10. Fatty acid profile (% total fatty acids) of fillets of European sea bass juveniles fed the experimental diets, before and after 3 days of storage.

Fatty acid	Storage Time (days)									
	0					3				
	HM0	HM7.5	HM15	HM22.5	SEM	HM0	HM7.5	HM15	HM22.5	SEM
C14:0	3.02	3.03	2.81	2.99	0.04	2.79	3.01	2.95	3.02	0.09
C16:0	15.57	15.4	15.34	15.16	0.08	15.4	15.36	15.39	15.2	0.07
C18:0	3.44	3.31	3.48	3.44	0.03	3.54	3.23	3.37	3.43	0.03
C16:1n-7	4.45^{b}	4.47^{b}	4.11^{a}	4.33^{ab}	0.04	4.2	4.45	4.3	4.35	0.04
C18:1n-9	18.66	18.84	18.64	18.85	0.2	18.19	18.94	19	19.21	0.21
C18:1n-7	2.17	2.14	2.09	2.14	0.01	2.12	2.14	2.14	2.15	0.01
C20:1n-9	2.01	2.05	1.93	2.03	0.02	1.94	2.04	2.02	2.04	0.02
C22:1n-11	1.63^{b}	1.60^{ab}	1.49^{a}	1.61^{ab}	0.02	1.56^{ab}	1.55^{a}	1.61^{ab}	1.67^{b}	0.02
C18:2n-6	8.52	8.75	8.56	8.65	0.08	8.22	8.73	8.88	8.71	0.09
C20:4n-6	1.63	1.55	1.67	1.57	0.02	1.80^{b}	1.60^{ab}	1.52^{ab}	1.48^{a}	0.03
C18:3n-3	1.41	1.45	1.43	1.42	0.02	1.36	1.45	1.49	1.43	0.02
C18:4n-3	1.02	1.02	0.93	1.02	0.01	0.96	1.01	0.98	1.03	0.01
C20:5n-3(EPA)	8.05	7.68	7.76	7.78	0.07	7.93	7.67	7.64	7.7	0.07
C22:5n-3	2.34	2.32	2.23	2.37	0.03	2.24	2.19	2.17	2.28	0.02
C22:6n-3(DHA)	20.01	19.1	20.58	19.9	0.23	21.82^{b}	20.10^{ab}	19.29^{a}	19.81^{ab}	0.34
Σ SFA	23.33	23.71	23.3	23.13	0.12	23.19	23.41	23.4	23.52	0.13
Σ MUFA	30.41	31.23	30.08	30.31	0.22	29.15	30.91	30.78	30.59	0.3
Σ PUFA	46.20	45.16	46.37	46.28	0.28	47.83	46.08	45.30	45.23	0.61
Σ n-6	11.44	11.76	11.74	11.7	0.07	11.39	11.79	11.89	11.75	0.07
Σ n-3	33.81	32.45	33.76	33.65	0.27	35.56^{b}	33.39^{ab}	32.50^{a}	32.53^{a}	0.39
Ratios										
n-3/n-6	2.99	2.77	2.91	2.87	0.03	1.92	1.73	1.82	2.01	0.05
PUFA/SFA	3.11	2.78	2.77	2.78	0.07	1.9	1.82	1.78	1.76	0.08

Table 10. Fatty acid profile (% total fatty acids) of fillets of European sea bass juveniles fed the experimental diets, before and after 3 days of storage (cont.).

	Two way ANOVA						
	Variation Source			**Diets**			
	Diet	**Time**	**Interaction**	**HM0**	**HM7.5**	**HM15**	**HM22.5**
C14:0	NS	NS	NS				
C16:0	NS	NS	NS				
C18:0	**	NS	NS	b	a	ab	ab
C16:1n-7	***	NS	***				
C18:1n-9	NS	NS	NS				
C18:1n-7	NS	NS	NS				
C20:1n-9	NS	NS	NS				
C22:1n-11	NS	NS	***				
C18:2n-6	NS	NS	NS				
C20:4n-6	**	NS	***				
C18:3n-3	NS	NS	NS				
C18:4n-3	*	NS	NS	ab	ab	a	b
C20:5n-3 (EPA)	NS	NS	NS				
C22:5n-3	NS	**	NS				
C22:6n-3 (DHA)	NS	NS	*				
Σ SFA	NS	NS	NS				
Σ MUFA	NS	NS	NS				
Σ PUFA	NS	NS	NS				
Σ n-6	*	NS	NS	a	b	b	ab
Σ n-3	***	NS	***				
Ratios							
n-3/n-6	NS	**	NS				
PUFA/SFA	NS	NS	NS				

Two-Way ANOVA: *p< 0.05; ** p < 0.01; *** p < 0.001; NS: non-significant; If interaction was significant, one-way ANOVA was performed for each diet and means in the same line with different superscript letters are significantly different (P<0.05). SEM: pooled standard error of the mean.

The fatty acids C12:0, C13:0, C15.0, C17:0, C20:0, C22:0, C24:0, C14:1n-5, C16:1n-9, C20:1n-11, C20:1n-7, C22:1n-9, C22:1n-7, C18:3n-6, C20:2n-6, C20:3n-6, C22:2n-6, C22:4n-6, C22:5n-6, C20:3n-3, C20:4n-3, C21:5n-3, C16:2n-4, C16:3n-4, C18:2n-4, C18:3n-4, C16:4n11, C18:4n-1, in percentage <1%, were also detected but not reported in the table for brevity. They were utilized to calculate the fatty acid groups.

Table 11. Nutritional quality indices of lipids in fillets of European sea bass fed the experimental diets, before and after 3 days of storage.

Index	Storage Time (days)									
	0					3				
	HM0	HM7.5	HM15	HM22.5	SEM	HM0	HM7.5	HM15	HM22.5	SEM
AI	0.371	0.374	0.358	0.367	0.003	0.355	0.372	0.366	0.371	0.004
TI	0.116	0.117	0.098	0.110	0.003	0.098	0.114	0.111	0.118	0.004
HH	3.258	3.276	3.396	3.349	0.023	3.391	3.301	3.321	3.318	0.028

	Two way ANOVA		
	Variation Source		
	Diet	Time	Interaction
AI	NS	NS	NS
TI	NS	NS	NS
HH	NS	NS	NS

NS: non-significant. SEM: pooled standard error of the mean.
AI, atherogenicity index; TI, Thrombogenicity index; HH, hypocholesterolemic/hypercholesterolemic FA ratio

Discussion

One of the main concerns of aquaculture production is the development of commercial, cost effective feeds using locally available, cheap and unconventional feed resources. For this reason, the search for sustainable ingredients alternative to FM, a limited resources available from the sea, has been a priority. Insect meal, which is part of the natural diet of fish, leaves a small ecological footprint and has a limited need for arable land, can be utilised to obtain meal that may represent a good candidate for aquafeeds (Henry et al., 2015). Insects possess many advantages such as fast reproduction, high protein content with an adequate EAA profile and the possibility of modulation of its nutritional quality through the adequacy of insects rearing conditions and diet.

BSF larvae has been widely studied as a FM substitute and has revealed the potential to be a partially or fully substitute of FM in fish diets. Also, it has been demonstrated in various fish species that there is a decrease in growth performance with increasing dietary levels of *Hermetia* meal (HM). This can be a result of decreased diet acceptability and feed intake. This normally happens when there is a substitution of substantial amounts of FA in a diet (Kroeckel *et al*., 2012). Still in rainbow trout (*Oncorhynchus mykiss*) it was possible to replace 25% of the FM in a diet without affecting growth and feed conversion ratio and it showed, at the same time, there is the possibility of decreasing fish oil supplementation due to *Hermetia illucens* high fat content (Sealey *et al*., 2011; St-Hilaire *et al*. 2007a). Also in studies performed in channel catfish (*Ictalurus punctatus*)it was highlighted that the use of BSF pre-pupae appears to be beneficial as a replacement for FM since despite the high chitin and fat content of BSF there were no adverse effects. In terms of quality, the aroma and texture of channel catfish fed chopped BSF larvae was acceptable for the consumer. However, the inclusion of more than 7.5% BSF meal seems unnecessary (Newton, 2004; Makkar *et al*, 2014). In Atlantic salmon (*Salmo salar*) fed diets in which FM was partially replaced by HM at 25%, 50% and 100%, there were no differences shown between fish fed the control diet and fish fed diets containing insect meal in performance, histology and sensory testing (Lock *et* al, 2014). Juvenile turbot (*Psetta maxima*) fed diets containing up to 33% defatted BSF prepupae meal showed no adverse effects on feed intake and feed conversion. Nonetheless, there was a low growth rate observed in this study in turbot fed diets with different inclusion levels of BSF. Turbot fed diets with inclusion levels higher than 33% revealed a decreasing of the protein digestibility, a reduced feed intake and a lower growth performance (Kroeckel *et al*, 2012).

To date, for European sea bass no studies were conducted to evaluate the potential of HM meal as a FM replacer. Nevertheless, there is a study conducted by Gasco *et al*., (2016) about other insect species included as meal in the diet, where it has been demonstrated that the addition of *Tenebrio molitor* to sea bass diets can be used up to 25% without compromising growth performance. Above this 25% level of inclusion there was a negative effect on the whole body FA profile. So sea bass is proven to accept diets with incorporation of insect.

There has been an increasing demand for sea bass, in Europe, due to its nutritional value and overall quality. In Italy, European sea bass represents half of the national eurialine fish farming product and it is in the list of the finfish species preferred by the Italian consumers (Fuentes *et al*., 2010; Poli *et al.,* 2001). Also, in Portugal it represents one of the most produced species in aquaculture (INE, 2010).This species is characterized by a lean muscle due to the fact that lipids are essentially store outside of locomotory muscle, in liver and adipose tissue (McClelland *et al*. 1995). Indeed, it was observed that the increase of dietary lipid level form 12 to 24% did not alter muscle lipid content but increased the visceral indixes and liver lipids content (Peres and Oliva-Teles, 1999). When diets with a traditional composition are utilised, muscle is composed by healthy quality lipids, as indicated by the high n-3 PUFA level, the low index of thrombogenicity, along with very low formation of peroxidation products during shelf life (Poli *et al*., 2001).

Fish flesh quality results from a series of complex characteristics such as chemical composition, texture and colour, among others, which are considered quality parameters. These parameters are affected by intrinsic (fish species, age, sex etc) and extrinsic factors (temperature, salinity, etc.) (Fuentes *et al*., 2010). In sea bass production feeding and rearing practices may alter nutritional value and organoleptic characteristics of fish, and in particular muscle fatty acid profile and fat concentration consequently having an influence on the flesh quality of fish (Testi *et al.*, 2006; Trocino *et al.*, 2012). This relation between flesh quality and feeding and rearing practices can be observed in the flesh of farmed fish which possess a higher lipid content than the wild fish of the same species. This is due to the fact that artificial diets provide a wide range of nutrients, which not only affect fish growth rate but also flesh composition, and especially lipid content that can be modified either quantitatively or qualitatively (Fuentes *et al*., 2010; Testi *et al.*, 2006).

There has been some research about FA substitution and its consequences on flesh quality parameters. Study performed with red sea bream fed diets with FM as well as

diets with other protein source revealed no change in flesh quality of fish (Aoki *et al*., 1996). However, studies with European sea bass fed diets with inclusion of freezed-dried microalgae resulted in a decreasing of lipid and a moderate reduction of n-3 PUFA in the muscle tissue (Tibaldi *et al*., 2015). Also, other researches on rainbow trout and sea bass revealed that the muscle of this specimens showed a higher peroxidation level when fed diets containing FM as the primary source of protein in comparison with fish fed diets with vegetables proteins which showed low levels of peroxidation (Lopez-Bote *et al*., 2001). This implies that dietary protein sources may alter the peroxidative characteristics of fish muscle (de Francesco *et al*., 2004).

To the best of our knowledge, data regarding the effects of FM substitution by HM on fish quality related parameters is limited. Thus it is necessary to gain deeper insight on the possible effect of HM inclusion in the diet on fish quality parameters, in order to guarantee that this product can enter the market without having a great impact on consumers' choice and human health. Therefore, the present study was conducted to investigate if the dietary increasing levels of HM may affect the quality parameters of European sea bass.

The obtained values of body morphological traits, were not significantly affected by the experimental diets, although in terms of body weight it seems that fish fed the diet HM7.5 had a higher body weight than those of fish fed the other diets. According to St-Hilaire *et al*. (2007a) rainbow trout weight gain was not affected by replacing 25% of HM in the diet, while on the other hand fish fed diet with 50% of replacement had lower weight gain than the control fish. Contrary to these results, in the growth trial performed by Sealey *et al*. (2011), results revealed that fish fed the 25% and 50% HM diet had lower weight gain in comparison with the control fish. Moreover, Kroeckel *et al*. (2012) found that with the increasing inclusion of HM in the diets, there was a decreasing of growth performance in turbot due to lower diet acceptability and feed intake. Overall in the present study, HM seemed to be a good protein source for European sea bass and may be used up to 22.5% of inclusion without any detrimental negative effects on the growth of fish.

As soon as the fish dies there are several *post-mortem* biochemical changes such as protein and ATP degradation, lower pH, lipid oxidation and unpleasant compound production by bacterial action which alters the fish quality and shelf life. Muscle is also subject to changes in water holding capacity, texture and colour (Ocanõ-Higuera *et al*., 2011).According to Poli *et al*. (2001) studies, sea bass stored at 4 ºC showed a 6 day

shelf life, thereafter fish were unfit for human consumption. For the present trial it was established a 3 day period of storage in a fridge at temperature +2/4 ºC to evaluate quality attributes and their changes in the first period of refrigerated storage.

After death in fish there is a decreasing in pH values, is due to the accumulation of lactic acid resulting from the glycolysis. Even though, in a state of rest prior to slaughtering there has been established a pH value lower limit of 6.2. pH value varies depending on the fish species (Love, 1988). Studies performed in sea bass revealed that during the storage of this species at + 4º C this species demonstrated a decreasing in the muscle pH from 6.9 to 6.5, after 24h of storage (Poli *et al*., 2001). Although in this study it was also observed a decrease in muscle pH values; storage time did not significantly affect pH values. However if a longer period of storage was considered it would be expected that pH values would increase due to microbial activity which is responsible for the decomposition of nitrogenated compounds (Hernández *et al*., 2009). An increase of pH during a prolonged storage was observed in Poli et al. (2003) in meagre where pH muscle dropping in 48 hour storage and then revealing and increasing pattern for the rest of the storage period.

Still, in this study there was a significant difference between experimental diets in which fish fed diet HM22.5 revealed and higher pH in comparison with fish fed the other diets.

From the point of view of consumer acceptance, colour is an important characteristic (Tibaldi *et al*., 2015). It is considered an indicator of health in aquaculture species and it is utilised to evaluate the quality of fishing products. Also, during storage time, the initial colour of the fish changes affecting the quality (Ocanõ-Higuera *et al*., 2011; Suárez *et al*., 2014). Some ingredients used on diet may possess pigments that can alter fish pigmentation of skin and flesh. As an example, Tibaldi *et al*. (2015) studied sea bass fed test diets with different inclusion levels of dried microalgae and found that fish suffered skin colour alteration by giving it a greenish pigmentation as well as a slightly yellowish flesh. Still in the sensory evaluation this was not a discriminatory factor (Tibaldi *et al*., 2015). Furthermore, Montero *et al*. (2005), reported a higher yellowness in flesh colouration of sea bass fed diets replacing fish oil with vegetable oil. On the contrary, in the present study, in colour of skin, fillet and liver, these differences between experimental diets were not significantly different. For this reason HM seemed to have no effect on colour of fish. Still, further researches are needed to confirm the results observed in this trial. It is important to evaluate this new dietary treatment in sea bass with sensory analyses to see if it influences certain quality attributes of the whole fish or of the flesh, in order to gather information from the point of view of the consumer

acceptance (Tibaldi *et al*., 2015). This sensory analysis was not possible in this study due to the fish small size, but it is important to get more knowledge about this subject. Also in this study no colour significant changes due to storage were detected in fillet analised at time T0 and time T3. According to Hernandez *et al*. (2009) studies on meagre, colour changes were detected on fillets starting day four and by day five colour intensity started to decrease on the flesh and skin. Similar results were obtained on the research led by Poli *et al*. (2006) in sea bass fillets.

In terms of fillet proximate constituents, with exception of ash which showed differences between fish fed control diet and fish fed HM diets, are not consistent with results from the literature available about HM. Studies still confirmed that body crude protein was not influenced by diet still, ash content was also not affected by diet. On the other hand crude lipid content was markedly influenced by the experimental diets (St-Hilaire *et al*., 2007a; Kroeckel *et al*., 2012).

FAs of fish lipids are highly unsaturated, for this reason, lipid components of fish muscle tissue are more likely to suffer oxidation (Foegeding *et al*., 1996). The occurrence of lipid hydrolysis and oxidation is determined by an increase of free FAs, peroxide and thiobarbituric acid reactive substances (TBARS) (Losada *et al*., 2005; Chaijan *et al*., 2006; Sae-leaw *et al*., 2013).Evidences indicate that during frozen storage, fish muscle composition as well as oxidative stability are affected by feed composition. This may be due to feed components that influence the deposition of antioxidants in the tissue altering its oxidative stability (Baron *et al*., 2009) Carotenoids, astaxanthin and canthaxanthin, that are pigments present in salmonids, may result as a defense by reducing oxidative susceptibility (Secci *et al.,* 2016). Simitzis *et al*. (2014) confirmed that the principal factor affectig lipid oxidation in sea bass is the storage length. Poli *et al*. (2001) revealed that sea bass stored at +4º C or +1 ºC with ice cover showed during the edibility period low oxidation index of lipids. In the present study lipid oxidation was evaluated trough measurements of primary (conjugated dienes) and secondary (malondialdehyde) oxidation products. Results showed that experimental diets and storage time significantly affected lipid oxidation. In fact fish fed HM diet showed less lipid oxidation in T3 than fish fed the control diet. Hence based on the available literature there is no explanation for the different results to diets and storage time since there has been very few investigations where lipid oxidation has been studied in fish fed diets with inclusion of insects, especially *Hermetia illucens* prepupae. It seems however, that chitosan, which is the deacetylated form of chitin, possess a number of useful properties for nutrition such as antimicrobial activity, the ability to form protective coatings, the binding action and antioxidant activity. Numerous studies have

indicated that chitosan may reduce lipid oxidation in fish and fish products (Georgantelis *et al*., 2007; Ojagh *et al*., 2010). Rainbow trout fillet with coating of chitosan and cinnamon oil was studied by Ojah *et al*. (2010) who found that this coating had a positive effect in the inhibition of lipid oxidation and microbial growth during refrigerated storage. In fresh pork sausages stored at +4 ºC, Georgantelis *et al*. (2007) observed that the combination of rosemary extract and chitosan used in sausages, resulted in lower value of malondialdehyde at the end of 20 days of storage period in comparison with the values obtained at day 5 in the controls. Also, in shrimp fed chitin and chitosan diets, Niu *et al*. (2013) found that MDA values were lower than those of shrimp fed other diets. For this reason the values of MDA in the present study may be related to the chitin present in HM utilised to formulate the experimental diets.

There are several parameters that can affect total lipid and FA composition, such as reproductive cycle period, size, sex and geographical location (Guil-Guerrero *et al*., 2010). There is a close relation between diet composition and flesh and liver FA composition. In most marine fish species the liver is the major site of lipid storage. Although some fish, like salmonids, accumulate FAs mainly in muscle tissues, lean fishes store the FAs in body cavities and perivisceral organs, like the liver (Guil-Guerrero *et a*l., 2010; Mourente *et al*., 2005). Like, sea bass in which has lean body is characterised by a preferential fat deposition in the liver (Poli *et al*., 2001). Liver is a key organ in detoxification and FA metabolism (Ferain *et al*., 2016). In this study, neither liver total lipids nor FA profile showed differences between experimental diets. Comparisons with other studies are difficult since the studies done on liver FA profile usually use diets with replacement of dietary vegetable oils.

Marine fish have a high requirement for diets containing high levels of n-3 FA especially n-3 HUFA such as EPA and DHA. Whereas, these FA are considered important for human nutrition, their concentration should be kept at optimum levels when testing a new diet for fish, considering that it may have an impact on FA composition (Mourente *et al*., 2005). As previously observed in other trials, in this study the dietary FA composition was reflected in the FA profile and composition of sea bass muscle tissue. The fatty acids C18:1 n9, C16:0 and C18:2 n6 were the most abundant FAs found in fillets of European sea bass, and these showed to be independent from the test diets which is in accordance with previous studies (Skalli and Robin, 2004).
In St-Hilaire *et al*. (2007a) in a trial on rainbow trout fed diets with 25% or 50% of BSF, the FA profile of muscle fillets showed lower values of the fatty acids (C14:0; C16:0;

C16:1n7; C18:0; C18:3n3; C18:4n3; C20:4n3; C20:5n3; C22:4n6; C22:5n3; C22:6n3) in fish fed diets with BSF, in comparison with fish fed the control diet. In terms of total lipids these were also lower in fish fed the experimental diets with BSF. Li *et al*. (2016) found increased values of SFA and DHA in the muscle of Jian carp fed diets with inclusion of BSF oil. This is contrary to what was observed in the present study in which, although there was an influence of the dietary FA composition in the FA profile of the tissue, there were not so many significant differences between fish fed the different experimental diets, at T0. Furthermore, data reported by Sealey *et al*. (2011) and by St Hilaire *et al*. (2007) showed that FA profile of rainbow trout fed diets containing BSF revealed decreasing levels of valuable n-3 PUFA, such as C18:3n3, C20:5n3 and C22:6n3, well known for their beneficial effects on humans health. These lower values of omega-3 FAs in fillets of fish fed BSF prepupae may be a consequence of lower levels of LC-PUFA in their diets (St-Hilaire *et al*., 2007b). Still, the values found in the present trial at T0 did not show significant differences in n-3 PUFA incidences in the groups of fish differently fed, maybe because the percentage of insect meal utilised by Sealey *et al*. (2011) and by St Hilaire *et al.* (2007a) were 25% or 50% much higher than the percentages of insect meal utilised in this trial (7.5%, 15% and 22.5%). Another reason for this could be that the fillets FA profile of fatty fish like, salmonids, eels, tuna and halibut, seemed to be more influenced by modification of fat source than those of lean and semi-lean fish (Turchini *et al*., 2009).

Nutritional quality of fillets can be evaluated by the n-3 PUFA contents and the AI and TI of fish muscle. The index of atherogenicity (AI) measures the capability to decrease blood lipid content and the index of thrombogenicity (TI) represents the tendency to reduce the formation of clots in the blood vessels (Grigorakis *et al*., 2007; Šimat *et al*., 2014). Fillets with better nutritional quality and have lower values of both these indices, consequently diets with low AI and TI values may reduce the potential risk of coronary heart disease (Hosseini *et al*., 2014).Data of previous studies on different sea foods nutritional quality showed that AI ranged from 0.33 to 2.37 and TI ranged from 0.01 to 1.18 (Filho *et al*., 2010; Kalogeropoulos *et al*., 2004; Rosa et al., 2007; Turan et al., 2007). According to the available literature, the value of AI was calculated to be 0.501 and 0.513 and TI 0.333 and 0.324, in cultured and wild sea bass respectively (Grigorakis et al., 2007). On the contrary, in the present study, both AI and TI had higher values than those seen in the literature for sea bass and were not significantly affected by diet or storage time. According to Poli *et al*. (2001) the evaluation of lipid quality by the indexes of AI and TI became worse as body weight and lipid content increases.

Also, another important nutritional index is the ratio between hypocholesterolaemic and hypercholesterolaemic FAs (HH), which is based on the effects (positive or negative) of certain FAs on cholesterol metabolism. A highest amount of HH ratio is the most desirable (Hosseini *et al*., 2014; Testi *et al*., 2006). Values of HH for different species found in previous studies range from 0.25 to 3.59 (Testi *et al*., 2006; Filho *et al*., 2010; Hosseini *et al*., 2014). Testi *et al*. (2006), in a experimental trial on sea bass, reported HH values of 2.18 and 2.03 for the dorsal portion and ventral portion, respectively. In this study HH values were higher than the ones reported by Testi *et al*. (2006) and were not significantly affected by the experimental diets or storage time.

Conclusions

Overall, the results of this study suggest that the replacement of FM by 7.5 to-22.5% *Hermetia illuceans* prepupae can be included in diets for short-term feeding of European sea bass without compromising colour, liver FA profile and nutritional index. Also, there was a decrease in the values of TBARS on fish fed HM diets which indicates that this ingredient may contribute to the decrease of lipid oxidation. According to this information HM may decrease the deleterious effect that are caused by lipid oxidation. Since insect meal is lacking of omega-3 in comparison with FM, when including this ingredient in the diet of farmed fish this should be take in consideration. Even though, in some studies about HM inclusion in diets have reported great differences; especially n-3 PUFA, in muscle FA profile of fish fed this diets in this study these differences were not so significant. According to these results, HM seems a suitable alternative protein source to FM in aquafeeds for European sea bass. Further research, are necessary to confirm the trend observed in this trial, including supplementary analyses to determine lipid oxidation and muscle FA profile, as well sensory analyses.

References

Andersen, U.B., Thomassen, M.S., Rora, A.M.B (1997). Texture properties of farmed rainbow trout (*Oncorhynchus mykiss*): Effect of diet, muscle fat content and time of storage on ice. Journal of the Science of Food and Agriculture 3, 347-353.

Aoki, H., Shimazu, H., Fukushige, T., Akano, H., Yamagata, Y., Watanabe, T., 1996. Flesh quality in red sea bream fed with diet containing a combination of different protein sources as total substitution for fish meal. Bull. Fish. Res. Inst. Mie. 6, 47–54.

Association of Official Analytical Chemists (2000). Official Methods of Analysis (17th ed.). Arlington, VA, USA: AOAC.

Ayoola, A.A. (2010). Replacement of fishmeal with alternative protein Source in aquaculture diets. Thesis Degree of Master of Science Faculty of North Carolina State University, North Carolina, USA.

Azhar, K. F., and Nisa, K. (2006). Lipids and their oxidation in seafood. J. Chem. Soc. Pakistan *28*, 298–305.

Baron, C. P., Hyldig, G., and Jacobsen, C. (2009). Does feed composition affect oxidation of rainbow trout (Oncorhynchus mykiss) during frozen storage? J. Agric. Food Chem. *57*, 4185–4194.

Barroso, F.G., de Haro, C., Sánchez-Muros, M.J., Venegas, E., Martínez-Sánchez, A., Pérez-Bañón, C. (2014). The potential of various insect species for use as food for fish. Aquaculture 422-423, 193–201.

Basurco, B., Lovatelli, A., García, B. (2011). Current status of Sparidae aquaculture. In Pavlidis, M.A., Mylonas, C.C., ed. (Sparidae: Biology and Aquaculture of Gilthead Seabream and Other Species, First edition), pp. 1–42.

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C.C., Paoletti, M.G., Ricci, A. (2013). Edible Insects in a food safety and nutritional perspective: A Critical Review. Compr. Rev. Food Sci. Food Saf. 12, 296–313.

Bondari, K., Sheppard, D.C. (1981). Soldier fly larvae as feed in commercial fish production. Aquaculture 24, 103–109.

Chaijan M., Benjakul S., Visessanguan W., Faustman C. (2006). Changes of lipids in sardine (Sardinella gibbosa) muscle during iced storage. Food Chemistry, 99, 83–91.

Cho, C.Y., Bureau, D.P. (2001). A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquat. Res. 32, 349–360.

CIE (Commission Internationale de L'Eclairage) (1976). Colorimetry. Bureau Central de la CIE, Vienna, Austria. Courtney.

de Francesco, M., Parisi, G., Médale, F., Lupi, P., Kaushik, S.J., Poli, B.M. (2004). Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*). Aquaculture 236, 413-429.

FAO (2012). The State of World Fisheries and Aquaculture 2012. Rome. 209 pp.

FAO (2009). Impact of rising feed ingredient prices on aquafeeds and aquaculture production. FAO Fisheries and Aquaculture Technical Paper, n. 541, 78 pp.

FAO (2014). The State of World Fisheries and Aquaculture. Opportunities and challenges. Rome, Italy, p.223.

FAO (2015). Cultured Aquatic Species Information Programme. Dicentrarchus labrax. Cultured Aquatic Species Information Programme. Text by Bagni, M. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 18 February 2005. [Cited 7 June 2016]. http://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax/en

FAO (2016).The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. Italy 200 pp.

Ferain, A., Bonnineau, C., Neefs, I., Rees, J. F., Larondelle, Y., Schamphelaere, K. A. C. De, and Debier, C. (2016). The fatty acid profile of rainbow trout liver cells modulates their tolerance to methylmercury and cadmium. Aquat. Toxicol. *177*, 171–181.

Filho M.M.R., Ramos M.I.L., Hiane P.A., Souza E.M.T.D. (2010). Nutritional value of seven freshwater fish species from the Brazilian pantanal. Journal of the American Oil Chemists' Society, 87, 1461–1467.

Foegeding E. A., Lanier T. C., Hultin H. O. (1996). Characteristics of edible muscle tissues. In O. R. Fennema (Ed.), Food chemistry, 880–942. New York, USA: Marcel Dekker, Inc..

Folch, J., Lees, M., Sloane-Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 496–509.

Francis, G., Makkar, H.P.S., Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197-227.

Fuentes, A., Fernández-Segovia, I., Serra, J.A., Barat, J.M. (2010). Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. Food Chem. 119, 1514–1518.

Gasco, L., Henry, M., Piccolo, G., Marono, S., Gai, F., Renna, M., Lussiana, C., Antonopoulou, E., Mola, P., and Chatzifotis, S. (2016). Tenebrio molitor meal in diets for European sea bass (Dicentrarchus labrax L.) juveniles: Growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. *220*, 34–45.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G.S., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac Res. 38, 551-579.

Georgantelis, D., Ambrosiadis, I., Katikou, P., Blekas, G., and Georgakis, S. A. (2007). Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 °C. Meat Sci. *76*, 172–181.

Grigorakis, K. (2007). Compositional and organoleptic quality of farmed and wild gilthead sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) and factors affecting it: A review. Aquaculture *272*, 55–75.

Guil-Guerrero, J. L., Venegas-Venegas, E., Rincón-Cervera, M. Á., and Suárez, M. D. (2011). Fatty acid profiles of livers from selected marine fish species. J. Food Compos. Anal. *24*, 217–222.

Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E. (2015). Review on the use of insects in the diet of farmed fish: Past and future. Anim. Feed Sci. Technol. 203, 1–22.

Hernández, M. D., López, M. B., Álvarez, A., Ferrandini, E., García García, B., and Garrido, M. D. (2009). Sensory, physical, chemical and microbiological changes in aquacultured meagre (Argyrosomus regius) fillets during ice storage. Food Chem. *114*, 237–245.

Hosseini H., Mahmoudzadeh M., Rezaei M., Mahmoudzadeh L., Khaksar R., Khosroshahi N.K., Babakhani A. (2014). Effect of different cooking methods on minerals, vitamins and nutritional quality indices of kutum roach (Rutilus frisii kutum). Food Chemistry, 148, 2014, 86–91.

Huss, H. H. (1995). Quality and quality changes in fresh fish. FAO fisheries technical paper (Vol. 348). Rome: FAO

INE/DGPA (2010). Estatísticas da Pesca. Lisboa: Instituto Nacional de Estatística. Lisboa, Portugal.

Kalogeropoulos N., Andrikopoulos N., Hassapidou M. (2004). Dietary evaluation of Mediterranean fish and molluscs pan-fried in virgin olive oil. Journal of the Science of Food and Agriculture, 84, 1750–1758.

Kaushik, S.J. (1998). Nutritional bioenergetics and estimation of waste production in non-salmonids. Aquat. Living Resour. 11, 211–217.

Klunder, H.C., Wolkers-Rooijackers, J., Korpela, J.M., Nout, M.J.R. (2012). Microbiological aspects of processing and storage of edible insects. Food Control 26, 628–631.

Kousoulaki, K., Sether, B.S., Albrektsen, S., Noble, C. (2015). Review on European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) nutrition and feed management: A practical guide for optimizing feed formulation and farming protocols. Aquac. Nutr. 21, 129–151.

Kroeckel, S., Harjes, A. G. E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., Schulz, C. (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.

Li, S., Ji, H., Zhang, B., Tian, J., Zhou, J., and Yu, H. (2016). Influence of black soldier fly (Hermetia illucens) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture *465*, 43–52.

Lie, Ø. (2001). Flesh quality – the role of nutrition. Aquac. Res. 32, 341–348.

Lock, E.J., Arsiwalla, T., Waagbø, R. (2014). Insect meal: a promising source of nutrients in the diet of Atlantic salmon (Salmo salar). In: Abstract Book Conference Insects to Feed The World, The Netherlands, 14–17 May, p. 74.

Lopez-Bote, C.J., Diez, A., Corraze, G., Arzel, J., Alvarez, M., Dias, J., Kaushik, S.J., Bautista, J.M., 2001. Dietary protein source affects the susceptibility to lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*) muscle. Anim. Sci. 73, 443–449.

Losada V., Piñeiro C., Barros-Velázquez J., Aubourg S. (2005). Inhibition of chemical changes related to freshness loss during storage of horse mackerel (Trachurus trachurus) in slurry ice. Food Chemistry, 93, 619–625.

Love, R. M. (1988). The food fishes. Their intrinsic variation and practical implications. London: Farrand Press.

Makkar, H.P., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1–33.

McClelland, G., Zwingelstein, G., Weber, J.-M., Brichon, G. (1995). Lipid composition of tissue and plasma in two mediterranean fishes, the gilt-head sea bream Chrysophyrys auratus and the European seabass (*Dicentrarchus labrax*) . Can. J. Fish. Aquat. Sci. 52, 161–170.

Montero, D., Robaina, L., Caballero,M.J., Gines, R., Izquierdo,M.S. (2005). Growth, feed utilization, flesh quality of European sea bass (Dicentrarchus labrax) fed diets including vegetable oils: a time course study on the effect of a re-feeding with a 100% fish oil diet. Aquaculture 248, 121–134.

Morrison, W.R., Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with born fluoride-methanol. Journal of Lipid Research 5, 600-608.

Mourente, G., and Bell, J. G. (2005). Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (Dicentrarchus labrax L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiv. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. *145*, 389–399.

Mourente, G., Dick, J.R. (2002). Influence of partial substitution of dietary fish oil by vegetable oils on the metabolism of [1-14C] 18:3n-3 in isolated hepatocytes of European seabass (*Dicentrarchus labrax* L.). Fish Physiol. Biochem. 26, 297–308.

Mourente, G., Tocher, D.R. (1993). Incorporation and metabolism of 14C-labelled polyunsaturated fatty acids in juvenile sea bream *Sparus aurata* L., in vivo. Fish Physiol. Biochem. 21, 443–453.

Newton, L., Sheppard, C., Watson, W., Burtle, G., Dove, R. (2004). Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Univ. of Georgia, College of Agric. & Environ. Sci., Dept. Of Anim. & Dairy Sci. Annual Report.

Nkafamiya, I.I., Osemeahon, S. A, Dahiru, D., Umaru, H. S. A. (2007). Studies on the chemical composition and physio-chemical properties of the seeds of baobab (*Adansonia digitata*). African J. Biotechnol. 6, 756–759.

Nordahl, P. (2011). Is the Aquaculture Industry Caught In a Fishmeal Trap? Thesis Degree of Science in Economics and Business Administration of NHH, Norway.

NRC (2011). Nutrient Requirements of Fish and Shrimp, Washington, D.C.: The National Academies Press.

Ocaño-Higuera V.M., Marquez-Ríos E., Canizales-Dávila M., Castillo-Yáñez F.J., Pacheco-Aguilar R., Lugo-Sánchez M.E., Graciano-Verdugo A.Z. (2009). Postmortem changes in cazon fish muscle stored on ice. Food Chemistry, 116 (4), 933–938.

Ojagh, S. M., Rezaei, M., Razavi, S. H., and Hosseini, S. M. H. (2010). Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chem. *120*, 193–198.

Oliva-Teles, A. (2000). Recent advances in European sea bass and gilthead sea bream nutrition. Aquac. Int. 8, 477–492.

Oliva-Teles, A., Enes, P., Peres, H. (2015). Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. Feed and Feeding Pratices in Aquaculture, 203-233.

Peres, H., Oliva-Teles, A. (1999). Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). Aquaculture 179, 325-334.

Peres, H., Oliva-Teles, A. (2002). Utilization of raw and gelatinized starch by European sea bass (Dicentrarchus labrax) juveniles. Aquaculture. 205, 287-299.

Peres, H., Oliva-Teles, A. (2007). Effect of the dietary essential amino acid pattern on growth, feed utilization and nitrogen metabolism of European sea bass (Dicentrarchus labrax). Aquaculture. 267, 119-128.

Poli, B. M., Messini, A., Parisi, G., Scappini, F., Vigiani, V., Giorgi, G., and Vincenzini, M. (2006). Sensory, physical, chemical and microbiological changes in European sea bass (Dicentrarchus labrax) fillets packed under modified atmosphere/air or prepared from whole fish stored in ice. Int. J. Food Sci. Technol. *41*, 444–454.

Poli, B. M., Parisi, G., Zampacavallo, G., Iurzan, F., Mecatti, M., and Bonelli, a (2003). Preliminary results on quality and quality changes in reared meagre (Argyrosomus. Aquac. Int. *11*, 301–311.

Poli, B.M., Parisi, G., Zampacavallo, G., Mecatti, M., Lupi, P., Gualtieri, M., Franci, O. (2001). Quality outline of European sea bass (*Dicentrarchus labrax*) reared in Italy: Shelf life, edible yield, nutritional and dietetic traits. Aquaculture 202, 303–315

Riddick, E.W. (2014). Insect protein as partial replacement for fishmeal in the diets of juvenile fish and crustaceans. In: Morales-Ramos, J., Rojas, G., Shapiro- Ilan, D.I. (Eds.), Mass Production of Beneficial Organisms. Invertebrates and Entomopathogens. Academic Press, San Diego, USA, pp. 565–582.

Rosa R., Bandarra N.M., Nunes M.L. (2007). Nutritional quality of African catfish Clarias gariepinus (Burchell 1822): A positive criterion for the future development of the European production of Siluroidei. International Journal of Food Science and Technology, 42, 342–351.

Sae-leaw T., Benjakul S., Gokoglu N., Nalinanon S., 2013. Changes in lipids and fishy odour development in skin from Nile tilapia (Oreochromis niloticus) stored in ice. Food Chemistry, 141, 2466–2472. Saint-Eve

Sánchez-Muros, M.J., Barroso, F.G., Manzano-Agugliaro, F. (2014). Insect meal as renewable source of food for animal feeding: A review. J. Clean. Prod. 65, 16–27.

Santos-Silva J., Bessa R.J.B., Santos-Silva F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. Livestock Production Science 77, 187-194.

Sapkota, A., Sapkota, A.R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., Lawrence, R. (2008). Aquaculture practices and potential human health risks: Current knowledge and future priorities. Environ. Int. *34*, 1215–1226.

Sealey, W.M., Gaylord, T.G., Barrows, F.T., Tomberlin, J.K., McGuire, M. A., Ross, C., St-Hilaire, S. (2011). Sensory Analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. J. World Aquac. Soc. 42, 34–45.

Secci, G., Borgogno, M., Lupi, P., Rossi, S., Paci, G., Mancini, S., Bonelli, A., Parisi, G. (2016). Effect of mechanical separation process on lipid oxidation in European aquacultured sea bass, gilthead sea bream, and rainbow trout products. Food Control 67, 75–81.

Sikorski, Z.E., Kalakowska, A. (2002). Chemical and Functional Properties of Food Lipids. Boca Raton, FL, CRC Press.

Šimat, V., Bogdanović, T., Poljak, V., and Petričević, S. (2015). Changes in fatty acid composition, atherogenic and thrombogenic health lipid indices and lipid stability of bogue (Boops boops Linnaeus, 1758) during storage on ice: Effect of fish farming activities. J. Food Compos. Anal. *40*, 120–125.

Simitzis, P. E., Tsopelakos, A., Charismiadou, M. A., Batzina, A., Deligeorgis, S. G., & Miliou, H. (2014). Comparison of the effects of six stunning/killing procedures on flesh quality of sea bass (Dicentrarchus labrax, Linnaeus 1758) and evaluation of clove oil anaesthesia followed by chilling on ice/water slurry for potential implementation in aquaculture. Aquaculture Research, 45(11), 1759–1770.

Skalli, A., Robin, J.H. (2004). Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (*Dicentrarchus labrax*) juveniles: Growth and fatty acid composition. Aquaculture 240, 399–415.

Stamer, A., Katz, P., Wessels, S., Hörstgen-Schwark, G. (2008). Protein concentrates derived from fly mass-production: *Hermetia*-meal as an alternative to fishmeal. 16th IFOAM Organic World Congress. Modena, Italy. June 16–20, 2008.

St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Sealey, W., Sheppard, C., and Irving, S. (2007b). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc. *38*, 309–313.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., Sealey, W. (2007a). Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 38, 59–67.

Suárez M.D., García-Gallego M., Trenzadoc C.E., Guil-Guerrerod J.L., Furné M., Domezaine A., Albae I., Sanz A. (2014). Influence of dietary lipids and culture density on rainbow trout (Oncorhynchus mykiss) flesh composition and quality parameter. Aquacultural Engineering, 63, 16–24.

Testi S., Bonaldo A., Gatta P., Badiani A. (2006). Nutritional traits of dorsal and ventral fillets from three farmed fish species. Food Chemistry, 98, 104–111. Thakur

Tibaldi, E., Chini Zittelli, G., Parisi, G., Bruno, M., Giorgi, G., Tulli, F., Venturini, S., Tredici, M.R., Poli, B.M. (2015). Growth performance and quality traits of European sea bass (*D. labrax*) fed diets including increasing levels of freeze-dried *Isochrysis* sp. (T-ISO) biomass as a source of protein and n-3 long chain PUFA in partial substitution of fish derivatives. Aquaculture 440, 60–68.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.I., Fontanillas, R., Nordgarden, U., Hevrøy, E.M., Olsvik, P., Berntssen, M.H.G. (2008). Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. Aquaculture 285, 193-200.

Tran, G., V. Heuzé, and H.P.S. Makkar. (2015). Insects in fish diets. Animal Frontiers 5:37-44

Trocino, A., Xiccato, G., Majolini, D., Tazzoli, M., Bertotto, D., Pascoli, F., and Palazzi, R. (2012). Assessing the quality of organic and conventionally-farmed European sea bass (Dicentrarchus labrax). Food Chem. *131*, 427–433.

Turan H., Sonmez G., Kaya Y. (2007). Fatty acid profile and proximate composition of the thornback ray (Raja clavata, L. 1758) from the Sinop coast in the Black Sea. Journal of Fisheries Sciences, 1, 97–103.

Turchini G.M., Bente E., Torstensen B.E., Ng W.N. (2009). Fish oil replacement in finfish nutrition. Reviwes in Aquaculture, 1, 10-17.

Ulbricht, T.L.V., Southgate, D.A.T., 1991. Coronary heart disease: seven dietary factors. Lancet 338, 985-992.

van Huis, A., Itterbeeck, J. Van, Klunder, H., Mertens, E., Halloran, A., Muir, G., and Vantomme, P. (2013). Edible insects. Future prospects for food and feed security.

Watters, C., Iwamura, S., Ako, H., Deng, D., Biosciences, M., Resources, H., and Feeds, A. (2012). Nutrition Considerations in Aquaculture: The Importance of Omega-3 Fatty Acids in Fish Development and Human Health. Foods Nutr. *FN-11*, UH – CTAHR 1–7.

White, K., O'Neill, B., Tzankova, Z. (2004). At a crossroads: will aquaculture fulfill the promise of the blue revolution? Silver Spring Meriland SeaWeb

Wu, D., Sun, D.W. (2013). Color measurements by computer vision for food quality control - A review. Trends Food Sci. Technol. 29, 5–20.