U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

MSc

2.º CICLO

FCUP
CIIMAR
UNIFI
2016

U.PORTO

EVALUATION OF THE POTENTIAL OF PREPUPAE MEAL OF BLACK SOLDIER FLY (Hermetia illucens) AS POTENTIAL INGREDIENT FOR RAINBOW TROUT (Oncorhynchus mykiss)

Ana Filipe Basto dos Santos

FC

U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

ciimar
Centro Interdisciplinar de Investigação Marinha e Ambiental

UNIVERSITÀ DEGLI STUDI FIRENZE
DISPAA
DIPARTIMENTO DI SCIENZE DELLE PRODUZIONI AGROALIMENTARI E DELL'AMBIENTE

EVALUATION OF THE POTENTIAL OF PREPUPAE MEAL OF BLACK SOLDIER FLY (*Hermetia illucens*) AS POTENTIAL INGREDIENT FOR RAINBOW TROUT (*Oncorhynchus mykiss*): EFFECT ON FLESH QUALITY

Ana Filipe Basto dos Santos

Dissertação de Mestrado apresentada à
Faculdade de Ciências da Universidade do Porto, Università degli Studi di Firenze
Mestrado em Recursos Biológicos Aquáticos

2016

MSc

EVALUATION OF THE POTENTIAL OF PREPUPAE MEAL OF BLACK SOLDIER FLY (*Hermetia illucens*) AS POTENTIAL INGREDIENT FOR RAINBOW TROUT (*Oncorhynchus mykiss*): EFFECT ON FLESH QUALITY

Ana Filipe Basto dos Santos
Mestrado em Recursos Biológicos Aquáticos
Departamento de Biologia
2016

Orientador
Doutora Helena Peres, Investigadora Auxiliar, CIIMAR

Coorientador
Doutora Giuliana Parisi, Professora Auxiliar e Investigadora, DISPAA

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

O Presidente do Júri,

Porto, _____/_____/_____

Acknowledgments

First of all, I want to express my enormous gratitude to **Prof. Dr. Aires Oliva-Teles** not only for accepting me as Master student of Biological Aquatic Resources and for accepting me in his laboratory (NUTRIMU) so friendly and readily, but also for giving me the opportunity to begin my journey in Fish Nutrition.

Secondly, I want to express my sincere gratitude to **Prof. Drª. Giuliana Parisi** not only for giving me this opportunity, for accepting to receive and guide me in her department (DISPAA) and shared with me her scientific knowledge, but also for her unconditional support, understanding and kindness throughout my incredible journey in Florence.

A huge and special thank you to **Valeria Iaconisi** for shared with me all her scientific knowledge, for having supported me at all times, especially in difficulty times, for her calm, effort, kindness, authenticity, honesty, readiness, among many other qualities.

I would like to thank to **Lorenzo Guerrini** for having given me to know a new work tool like ImageJ software and for his huge effort to understand my doubts and help me.

A big thank you to **Rita Pupino**, **Claudia Scarpaleggia**, **Monica Borgogno**, **Giulia Secci**, and **all members of DISPAA**, for having supported me whenever I needed.

Almost the last but not the least, I would especially like to express my enormous gratitude and admiration to **Prof. Drª. Helena Peres**, without doubts the main driving force of my work, not only for accepting me as her Master Thesis student so friendly and readily, but also for shared with me her scientific and non-scientific knowledge, for her tremendous support, encouragement, dedication, understanding and kindness throughout these last years, among many other qualities that make her an incredible professional and a wonderful person.

Finally, I would like thanks to my **family**, **friends** and to everyone who, in some way, contributed to the success of this thesis.

Abstract

Fishmeal and fish oils are still the main sources of proteins and lipids in aquafeeds. The decrease of global availability and high price of these resources have challenged industry to identify more sustainable alternatives. The potential of insects has been recognized recently. Insects may represent a good candidate as alternative protein source to fishmeal. Over the last decade, studies of the replacement of fishmeal with insect meal in the diet for fish have emerged and the promising results have encouraged further research. Black soldier fly (*Hermetia illucens*) larvae are a high-value feed source. Rainbow trout (*Oncorhynchus mykiss*) is the leading cultured freshwater fish species in Europe However, rainbow trout overproduction, associated with increasing price of feeds, led to a decrease in profitability of the intensive production of this species. Therefore, the present study aimed to contribute for the development of more sustainable aquafeeds for *O. mykiss*. For that purpose the effect of dietary partial replacement of fishmeal by prepupae meal of *H. illucens* in final product quality was evaluated. Thus, a trial was performed to testing the effect of dietary fishmeal replacing levels (0, 25 and 50%) by prepupae meal of *H. illucens* in quality traits of *O. mykiss* flesh. Furthermore, the effect of storage time and cooking process in quality traits of flesh of *O. mykiss* were also tested. Overall, results indicate that, irrespectively the dietary replacement level of fishmeal by *H. illucens* meal, no detrimental effects on morphometric, marketable and physical characteristics, including colour, pH, texture and water-holding capacity of fish flesh, were reported. Nonetheless, increasing inclusion of *H. illucens* meal led to a increase of cooking loss values, while proximate composition of raw and cooked fillet were not affected by the diet. Contrarily, flesh fatty acid profile, and consequently the quality indexes TI, AI, HH, PUFAn3/PUFAn6 and PUFA/SFA, were strongly affected by the substitution of fishmeal with insect meal. Saturated fatty acids increased with the inclusion of *H. illucens*, while MUFA and LC-PUFA (particularly EPA and DHA) decreased with the inclusion of insect meal in the diet. Interestingly, the muscle lipid oxidation products decreased with the inclusion of *H. illucens* in the diet. As expected, the increase of the storage time had a negative effect on almost all tested variables. Though, further studies are required to enhance the nutritional profile of *H. illucens* meal, especially those related to the fatty acid profile. Overall, it can be concluded that the dietary fishmeal replacement by *H. illucens* up to 50% had little effect on the quality traits of rainbow trout. However, an increase of saturated and a decrease of MUFA and LC-

PUFA were observed in muscle of fish fed the insect based diets, which results in a decrease of lipid peroxidation.

Keywords: Sustainable aquafeeds; *Hermetia illucens* meal; *Oncorhynchus mykiss*; Flesh quality.

Resumo

A farinha de peixe e o óleo de peixe são as principais fontes proteicas e lipídicas em dietas para aquacultura. A diminuição da disponibilidade global e o elevado preço destes recursos tem desafiado a indústria a identificar alternativas mais sustentáveis. O potencial dos insetos tem sido reconhecido recentemente. Os insetos podem representar um bom candidato como fonte de proteína alternativa à farinha de peixe. Ao longo da última década, têm emergido estudos sobre a substituição da farinha de peixe por farinha de inseto nas dietas para peixe e os seus resultados promissores têm encorajado mais pesquisas. As larvas de mosca negra (*Hermetia illucens*) são uma fonte alimentar de elevado valorA truta arco-íris (*Oncorhynchus mykiss*) é a principal espécie de peixes de água doce produzida na Europa. No entanto, a sobreprodução associada ao aumento do preço das dietas, levou a uma diminuição da rentabilidade da produção intensiva desta espécie. Neste sentido, o presente estudo tem como objetivo contribuir para o desenvolvimento de dietas para aquacultura de *O. mykiss* mais sustentáveis. Desta forma, o efeito da substituição parcial, na dieta, da farinha de peixe por farinha de pré-pupa de *H. illucens* na qualidade final do produto foi avaliado. Assim, foi realizado um ensaio para testar o efeito de diferentes níveis de substituição (0, 25 e 50%) de farinha de peixe por farinha de pré-pupa de *H. illucens* nas características de qualidade do músculo de *O.mykiss*. O efeito do tempo de armazenamento e do processamento na qualidade do músculo de *O. mykiss* foram também testados. Na generalidade, os resultados indicam que, independentemente do nível de substituição de farinha de peixe por farinha de inseto na dieta, não foram produzidos efeitos prejudiciais sobre as características morfométricas, de comercialização e físicas, incluindo cor, pH, textura e capacidade de retenção de água do músculo do peixe. No entanto, o aumento da inclusão de *H. illucens* na dieta levou a um aumento dos valores de *cooking loss*, enquanto a composição centesimal dos filetes crus e cozidos não foram afetados pela dieta. Contrariamente, o perfil de ácidos gordos dos filetes e, consequentemente, os índices de qualidade TI, AI, HH, PUFAn3/PUFAn6 e PUFA/SFA, foram fortemente afetados

pela substituição da farinha de peixe por farinha de inseto. Os ácidos gordos saturados aumentaram com a inclusão de *H. illucens*, enquanto os *MUFA* e *LC-PUFA* (particularmente EPA e DHA) diminuíram com a inclusão de farinha de inseto na dieta. Curiosamente, os produtos de oxidação de lípidos no músculo diminuíram com a inclusão de *H. illucens* na dieta. Como esperado, o aumento do tempo de armazenamento teve um efeito negativo sobre quase todas as variáveis testadas. Não obstante, mais estudos são necessários para melhorar o perfil nutricional de *H. illucens*, especialmente estudos relacionados com o perfil de ácidos gordos. Concluindo, a substituição da farinha de peixe na dieta por pré-pupa de *H. illucens* até 50% teve pouco efeito sobre as características de qualidade da truta arco-íris. No entanto, foi observado um aumento dos ácidos gordos saturados e uma diminuição de *MUFA* e *LC-PUFA* no músculo dos peixes alimentados com as dietas à base de insecto, o que resulta numa diminuição da peroxidação lipídica.

Palavras-chave: Dietas sustentáveis para aquacultura; Farinha de *Hermetia illucens*; *Oncorhynchus mykiss*; Qualidade dos filetes.

Contents

Tables list

Figures list

Appendix List

Abbreviations

AA – Amino acid

AI – Atherogenicity index

BSF – Black soldier fly

CD – Conjugated dienes

CF – Condition factor

CL – Cooking loss

CT – Conjugated trienes

DHA – Docosahexaenoic acid

DISAFA – Dipartimento di Scienze Agrarie, Forestali e Alimentari

DISPAA – Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente

DM – Dry matter

DO – Dissolved oxygen

DY – Dressed yield

EAA – Essential amino acid

EFA – Essential fatty acid

EFSA – European Food Safety Authority

EPA – Eicosapentaenoic acid

EU – European Union

FA – Fatty acid

FAA – Free amino acid

FAME – Fatty acid methyl esters

FM – Fish meal

FO – Fish oil

FY – Fillet yield

HH – Hypocholesterolemic/hypercholesterolemic fatty acids ratio

HI – *Hermetia illucens*

HPP – High-pressure processing

LC- PUFA – Long chain polyunsaturated fatty acid

M€ – Million Euros

MCFA – Medium-chain fatty acid

MCT – Medium-chain triacylglycerol

MT – Million tonnes

MUFA – Monounsaturated fatty acid

PAP – Processed animal protein

PUFA – Polyunsaturated fatty acid

TAA – Total amino acid

TBA – Tertiary butyl alcohol

TBARS – Thiobarbituric acid-reactive substances

TCA – Thiobarbituric acid

TEP – Tetraethoxypropane

TI – Thrombogenicity index

UK – United Kingdom

WB – Warner-Bratzler

WHC – Water-holding capacity

Introduction

The state of the world aquaculture

More than 800 million people in the world still suffer from chronic malnourishment and the global population is expected to grow more 2 billion (30%) in the following years, to reach 9.7 billion people in 2050 and 11.2 billion in 2100. On these grounds, feed our planet while safeguarding its natural resources for future generations it's a challenge that has gained increasing importance. Thence, fisheries and aquaculture can play a significant role in eliminating hunger, promoting health and reducing poverty (FAO, 2014; FAO, 2016a; United Nation, 2015).

Since 1980s capture fishery production has been relatively stagnant and 31.4% of fish stocks were estimated as fished at a biologically unsustainable level and therefore overexploited by the fishery industry in 2013 (FAO, 2016a). Consequently, aquaculture plays now, more than ever, an important role in the fish supply for human consumption and remains one of the fastest-growing food producing sectors (FAO, 2016a). In 1974, aquaculture provided only 7% of fish for human consumption, in contrast with almost 50% in 2014 (**Fig. 1**) (FAO, 2016a).

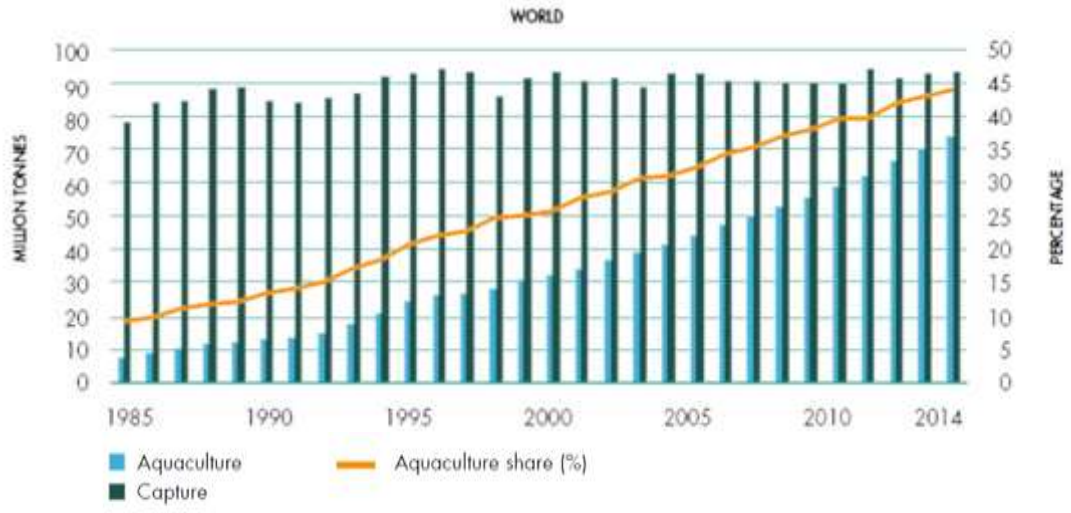

Fig. 1. Share of aquaculture in total production of aquatic animals. Source: FAO (2016).

According to FAO (1988), aquaculture is "the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc." Historically, world fish farming was first practiced in 2000 b.C., in China. One of the most significant progresses was the

development of culture methods for trout, but a revolution occurred in the 1970s led by development of new technologies and better feeding methods (FAO, 1998).

In 1990, world aquaculture production of fish contributed to 13.1 million tonnes (MT) (13.4%) of total fish produced (including products for non-food uses) in this year by capture fisheries and aquaculture production (FAO, 2016a). In 2000 accounted to 25.7% (32.4 MT), in 2010 to 39.8% (59.0 MT) and in 2014 fish gathered from aquaculture amounted to 73.8 MT (44.1%), with an estimated first-sale value of 143.4 billion Euros (**Fig. 1**) (FAO, 2016a). Indeed, the contribution of the aquaculture production to the total fish produced has greatly increased. All continents have shown a general trend of an increasing share of aquaculture production in total fish production, except Oceania where this share has decreased in the last three years (FAO, 2016a). Nevertheless, Asia has played a major role in this growth, accounted for 65.6 MT or more than 88% of global fish production from aquaculture in 2014 (FAO, 2016a).

In spite of the positive growing trend, it appears that aquaculture production is stagnating due its slower expansion in the period 2000-2012 (6.2%) than in the periods 1980–1990 (10.8%), 1990–2000 (9.5%) and 2000–2010 (5.8%) (FAO, 2016a). Although at a slowing rate, these results reflects that the global trend of aquaculture development gaining importance in total fish supply has remained uninterrupted and world aquaculture production continues to increase, playing an important role in world food production (FAO, 2016a; Walton et al., 2015).

The state of the European aquaculture

Europe is one of the world's main seafood consumers, representing the largest market for fish in the world (STECF, 2014). Due to the stagnation, or even decrease, of capture fisheries and slow growth rate of aquaculture production, fish imports has increased in the European Union (EU), where 65% of the seafood consumed was imported, in the year 2014 (STECF, 2014). Consequently, the increasing demand offers an unique opportunity to expand the aquaculture production in the EU.

In 2013, the EU was the fourth largest fisheries and aquaculture producer worldwide, with a volume of 1.30 MT of aquaculture products, accounting for 3.22% of total world fishery and aquaculture production (EUMOFA, 2015). According to the most recent data from FAO (2016a), in 2014 this volume increased to 2.90 MT of aquaculture products (excluding aquatic plants and non-food products), accounting for

3.97% of total world production (always excluding aquatic plants and non-food products) and almost 18% of the total European production (including non-food products) (**Fig. 2**). Though, this data are provisional and subject to revisions.

In 2012, the main countries that contributed to the EU aquaculture were France, German, Italy, Spain and the United Kingdom, accounting for almost 80% in volume of total EU aquaculture production (EUMOFA, 2015).

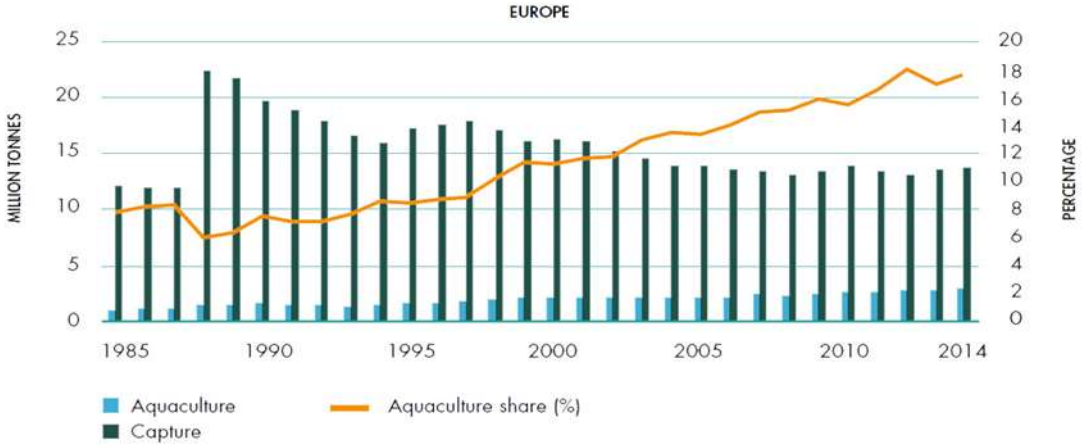

Fig. 2. Share of the European aquaculture in total production of aquatic animals. Source: FAO (2016a).

The aquaculture production in EU can be divided into Marine, Shellfish and Freshwater production, accounting for 31, 48 and 21% of the EU aquaculture production in quantity, respectively. In terms of value, the marine species accounted for 53% of the EU aquaculture production, the freshwater species accounted for 19% and shellfish for 28% (STECF, 2014). In 2012, the main species produced in EU in terms of volume were Mediterranean mussel (328 thousand tonnes), Atlantic salmon (179 thousand tonnes), Pacific cupped oysters (138 thousand tonnes) and rainbow trout (134 thousand tonnes), accounting for 26, 14, 11 and 11% of total EU production, respectively (STECF, 2014). On the other hand, in terms of value, the main species produced in EU were: Atlantic salmon (782 million Euros (M€)), Pacific cupped oysters (633 M€), European seabass (455 M€), gilthead seabream (433 M€) and rainbow trout (416 M€), accounting for 20, 16, 11, 11 and 10% of total EU aquaculture value, respectively.

The Portuguese aquaculture produced 10 791 t in 2014, while the total value was 50.3 M€, which corresponded to an increase of 7.2% in volume, but to 8.3% decreased in revenue when compared to the years 2013. This result is justified by the reduction of the average sale prices of some species, namely turbot (INE, 2016).

Rainbow trout (*Oncorhynchus mykiss*, W.)

Habitat and biology

Oncorhynchus mykiss (Walbaum, 1792) **(Fig. 3)** is a salmoniform fish that belongs to Salmonidae family. The body of *O. mykiss* is elongate and fusiform, laterally compressed with the adipose fin proximate to caudal peduncle. Males can be distinguished from females by head shape, the males usually have a hooked jaw and females have a more curved operculum, during the reproductive period. The overall body colour is blue to olive green, with a pink band along the lateral line, and silver below. This species is also characterized by having the whole body covered with small black spots and particularly located in caudal and adipose fins. Though, the body coloration varies with habitat, size and sexual condition. Anadromous and spawners are darker and have more intense colours. On the other hand, freshwater residents are lighter, brighter, and more silvery (FAO, 2016b; FishBase, 2016).

Fig. 3. Rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792). Source: FAO (2016b).

This species is native to the Pacific drainages of North America, ranging from Alaska to Mexico. Since 1870, it was introduced on all continents, except Antarctica, for recreational angling and aquaculture purposes. Thus, today is the most widely introduced salmonid worldwide and one of the most extensively introduced fish species in general (Stanković et al., 2015). *Oncorhynchus mykiss* is capable of occupying many different habitats, ranging from an anadromous life history to permanently inhabiting lakes. The anadromous strain is known for its rapid growth, achieving 7-10 kg within 3 years, whereas the freshwater strain can only achieve 4.5 kg. The species can tolerate vast ranges of temperature variation (0-27 °C), but spawning and growth occurs in a thinner range of 9-14 °C. This species can be found in depths between 0 and 200 meters (FAO, 2016b; FishBase, 2016).

The spawning of *O. mykiss* occurs from November until May, in the North hemisphere and from August to November, on the South hemisphere. Females are able to produce up to 2000 eggs/kg of body weight. In the wild, shortly after emergence, young fish move downstream at night and remain in relatively shallow areas, whereas adults can reach deeper waters. *O. mykiss* is mainly a carnivorous species, feeding of terrestrial and aquatic invertebrates. Insects remain their dominant food source throughout life, in the wild. However, they are also considered opportunist feeders, since they can adapt their diets according to local feed availability, accessibility and temporal variation. In the wild specimens, the consumption of freshwater shrimp, containing the carotenoid pigments, is responsible for the orange-pink colour of their flesh (FAO, 2016b; FishBase, 2016).

European aquaculture

France was one of the first countries that introduced *O. mykiss* in Europe. The first translocation to Europe dates back to 1879, when Trocadero Aquarium in Paris received eggs of this species from the US Fish Commission and raised them successfully. Though, the first land-based rainbow trout production enterprise in Europe was established in Denmark, where instead of following the common practice of releasing the young fish to enhance the local watersheds, they continued to keep and fed the fish in captivity (Stanković et al., 2015). Other countries followed suit, but many of their hatcheries did not have the additional water capacity for farm production. During the subsequent years, the rainbow trout production industry in Europe was dominated by Denmark (Nash, 2011).

Since 1950s, the production of *O. mykiss* increased exponentially and, nowadays, this species is farmed practically in all the European countries. In 2012 the total volume of EU freshwater aquaculture was 312 thousand tonnes, generating a value of 991 million Euros. In this year, the European total production of rainbow trout was near 134 thousand tonnes, valued 416 million Euros. Therefore, in terms of weight, rainbow trout dominate this segment with 48% of the volume and 43% of the value (Fig. 4). The most important producers in terms of volume are Denmark (31%), France (24%), Spain (13%) and United Kingdom (11%) and the most important producers in terms of value are Italy (28%), France (18%) and Denmark (14%) (STECF, 2014).

In Portugal, in 2014 aquaculture production of rainbow trout account to 772 tonnes, being the sixth most produced aquaculture fish species, after, turbot, clams, gilthead seabream, oyster and European seabass (INE, 2016).

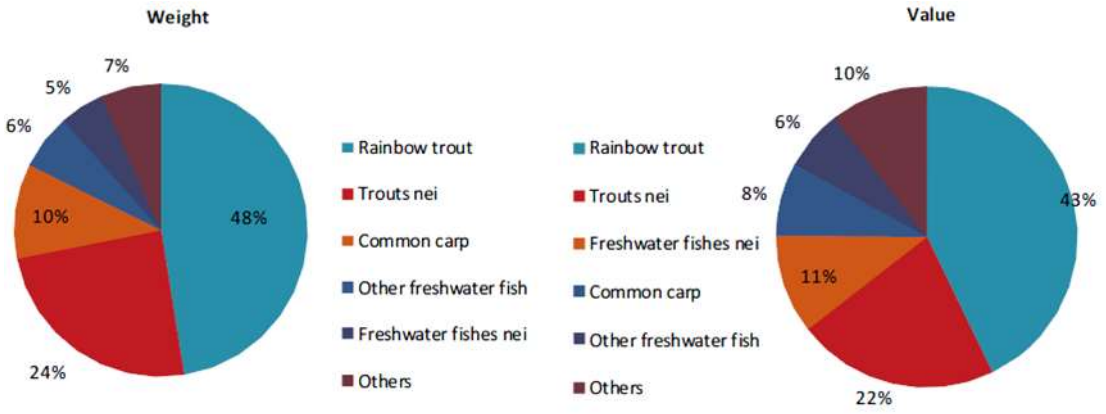

Fig. 4. Main species, produced in the EU member states excluding land lock countries freshwater farming facilities: Data referred to the year 2012. Source: STECF (2014).

Nutritional requirements

Oncorhynchus mykiss is one of the main important cultivated fish species with their nutrition requirements better described. This species requires ten essential amino acids, and the optimum dietary essential amino acid pattern was already determined, being lysine, methionine and arginine the first, second and third most limiting amino acids in rainbow trout feeds. The optimum ratio among essential and non-essential amino acid was also estimated to be 55:45 (Green et al., 2004). In practical terms, sufficient protein has to be given to ensure maximum growth performance and optimal health status (Oliva-Teles, 2012). This optimum protein level depends on fish size, water temperature, activity level, reproductive status, dietary energy level and other variables. Nevertheless, rainbow trout feeds generally are formulated to contain between 42 and 48% of crude protein, depending upon life-history stage (Hardy, 2002). Rainbow trout, as other vertebrates, require dietary sources of n3 fatty acids and this requirements are most effectively satisfied by eicosapentaenoic acid (EPA) (C20:5n3) and docosahexaenoic acid (DHA) (C22:6n3) because *O. mykiss* have a limited capacity to convert them from linolenic acid (C18:3n3) or stearidonic acid (C18:4n3) and thereby to produce EPA or DHA from the shorter-chain precursors (FAO, 2016c). Based on total body and tissue fatty acid composition data, the estimated EFA requirement of *O. mykiss* is 0.5-1.0% of the diet, depending on dietary lipid level and

life-history stage (Hardy, 2002; NRC, 2011). This species also requires small amount of arachidonic acid (C20:4n3) for phospholipid and prostaglandin synthesis (Knight and Rowley, 1995).

Rainbow trout do not have a specific requirement of dietary carbohydrates. However, there is a limit to the capacity of *O. mykiss* to tolerate high dietary carbohydrate levels when fed over an extended period. The optimum/maximum digestible carbohydrate level for rainbow trout is 15–17% (FAO, 2016c).

Regarding to vitamins, this species require 15 (vitamin A, B_{12}, D, E, K, thiamine, riboflavin, pyridoxine, niacin, choline, inositol, biotin, and ascorbic, folic and pantothenic acids), although the quantitative dietary requirements are not necessarily exact for some. Absolute dietary requirements for vitamins in fish are difficult to establish without precise clinical response variables that accurately can measure the effects of vitamins on metabolism and organism health. A dietary level sufficient to prevent the appearance of clinical signs of deficiency may not necessarily be the optimal one for all cells or tissues (Lie, 2001). Hence, it is prudent to slightly over fortify diets with vitamins, not only to avoid the loss of vitamin activity associated with diet processing and storage (Oliva-Teles, 2012), but also to account for cellular needs that may not be known yet (FAO, 2016c).

The mineral requirements of rainbow trout are poorly studied. The most mineral requirements for this species were identified by specific clinical signs of deficiency that resulted from inadequate dietary levels or from antagonistic interactions in feeds that reduced the bioavailability of minerals. Thus, adequate dietary levels of certain minerals should be taken into account due to interactions among feed ingredients, due to the presence of some anti-nutritional components and due to the low quantities of some of them in freshwater (Oliva-Teles, 2012; Hardy, 2002; NRC, 2011).

The importance of new ingredients in aquaculture feeds

Aquaculture, like any other food producing sector, is totally dependent on the provision and supply of nutrient inputs (Tacon and Metian, 2008). In the case of fish production, these nutrients are supplied through the consumption of natural food organisms produced within the culture system (extensive aquaculture production systems) or through the use of exogenous feed inputs, which may include the use of

natural food organisms, the use of farm-made or industrially compounded aquafeeds (semi-intensive or intensive aquaculture production systems) (Tacon and Metian, 2008).

Currently, aquafeeds represents 40–70% of the total costs of fish production (Rana et al., 2009) and they are especially high in the intensive aquaculture production, particularly of carnivorous fish species, like the rainbow trout (*Oncorhynchus mykiss*), that would require a relatively large amount of high quality fishmeal (FM) and fish oil (FO) (Henry et al., 2015; Magalhães et al., 2015).

According to FAO (2016a), fishmeal is "the crude flour obtained after milling and drying fish or fish parts", while fish oil is "usually a clear brown/yellow liquid obtained through the pressing of the cooked fish", both "produced from whole fish, remains or other fish by-products resulting from processing", and they are the favourite ingredients in industrially aquafeeds, constituting their majors dietary protein and lipid sources. FM allows quick fish growth and optimal feed conversion (Gao et al., 2013). It has a high protein content, high nutrient digestibility and palatability, balanced amino acid profile, lack of anti-nutritional factors and it is generally widely available (Barroso et al., 2014; Gatlin et al., 2007). It is also a highly tradable product, and its production can be an important source of revenue for some countries (FAO, 2016a). On the other hand, FO represents the preferred available source of long-chain highly unsaturated fatty acids (HUFAs), important in human diets for diverse critical functions of organism (FAO, 2016a).

In 2014, worlds FM and FO were produced from 76% (15.8 MT) of remains of captured fish and a significant proportion derived directly from capture fisheries of small pelagic fish, namely *anchoveta*, the main groups of species utilized. Peru and Chile are the main producing countries, responsible for 2/3 of the trade. Though, these countries are affected by El Niño phenomena and the catches are bound to fluctuate, consequently affecting the supply (FAO, 2016a).

The firm decline in catches of wild fish throughout the world, the fluctuations of capture of small pelagic fish, like *anchoveta*, due to El Niño phenomena, and the increased demands for livestock and aquaculture feeds have resulted in a fast decrease in the availability of FM and FO and in the simultaneous their price increase (Olsen and Hasan, 2012; Henry et al., 2015). Indeed, the development of alternatives for FM and FO in aquaculture diets is a crucial priority to ensure the economic and environmental sustainability of aquaculture over the long term (Barroso et al., 2014;

Glencross et al., 2007). According to Gatlin et al. (2007), an appropriate substitute for FM should be widely available at a competitive price, easy to handle, ship, store and incorporate in fish diets. In terms of nutritive value, it should be low in fiber and carbohydrates, high in protein, with a balanced amino acid profile, as well as be highly digestible and palatable.

Plant protein ingredients have been introduced in aquaculture fish diets in the attempt to replace FM (Médale et al., 2013; Henry et al., 2015). Though, the potential presence of anti-nutritional factors, the low concentrations of sulfur amino acids, the low palatability, the low/moderate digestibility and the potential problems of the inflammation of the digestive tract associated to high plant protein based diets are of concern (Médale et al., 2013; Henry et al., 2015). Further, the productions of FM or plant feedstuffs are the main contributors to land occupation, primary production use, acidification, climate change, energy use and water dependence (Sánchez-Muros et al., 2014).

According to Gasco et al. (2015), the idea of using insects in animal feed is not new. In 1919 Linder (1919) suggested that domestic fly could be created in organic wastes for production of proteins and fats for animal feed. Later, Calvert and Martin (1969) studied the use of housefly as feed for chickens, while in 1977, Newton et al. and his staff tried to use *Hermetia illucens* larvae as a food supplement in swine diets. In the early 1980s, due to the high availability of other protein sources, this unconventional raw material was set aside. However, nowadays as the situation regarding the availability and sustainability of raw materials used in animal feed has changed, new doors are open for these highly promising ingredients. However, it is necessary to extend knowledge through applied research (Gasco et al., 2015).

Insects as an innovative nutrient source in fish diet

Since insects are part of the natural diet of fish, they have been considered as potential alternatives to FM and FO. Their utilization as a protein and lipid source for fish feed has been hardly studied and the promising obtained results have encouraged further research (Henry et al., 2015; Sanchéz-Muros et al., 2014). Insects grow and reproduce easily, have high feed conversion efficiency as they do not use metabolic energy to maintain a constant body temperature, need a fewer arable land, and are expected to use less water in comparison with conventional feed resources, such as

soymeal and FM (Makkar et al., 2014; Nowak et al., 2016; van Huis et al., 2013). Some insects, like *Hermetia illucens*, can be reared on bio-waste reducing environmental contamination and transforming waste into high protein feed that can replace increasingly more expensive compound feed ingredients, such as fish meal (Sánchez-Muros et al., 2014). Furthermore, the waste from insects rearing can be used as an organic fertilizer, resulting in a closed circle principle (Rumpold and Schlüter, 2013). Thus, from an environmental point of view, insect production may be consider sustainable having relative low scores on carbon footprint meal (Sánchez-Muros et al., 2014). Besides, insects can be reared under different conditions to optimize their nutritive value. One kilogram of insect biomass can be produced from on average 2 kg of feed biomass (Tran et al., 2015). From the nutritional point of view, depending on species and/or stage, insects are rich in protein, lipids, vitamins and minerals (Barroso et al., 2014; Henry et al., 2015). Furthermore, some insects have antifungal activity and/or antibacterial peptides that may increase the shelf-life of insect-containing feeds (Henry et al., 2015; Ravi et al., 2011).

To incorporate any insect species into the diet of a fish species, it is obligatory to determine the exact composition of the insect, which varies according to its particular life stage, rearing conditions and diet, and to compare it with the nutritional requirements of the fish species of interest (Henry et al., 2015).

The protein requirements of different fish species range between 28-55% of dry diets. However, fish has no absolute dietary protein requirement *per se*, requiring a quantitatively and qualitatively balanced mixture of essential amino acids (EAA) and non-essential amino acids (Oliva-Teles, 2012). The AA profiles of most insects studied have shown a good correlation with fish requirement values, but the profile of Diptera has been considered as the closest to that of FM (Henry et al., 2015).

According to Oliva-Teles (2012), as the carbohydrate utilization by fish is not very efficient, lipids are the main energy source in fish diets and also are a source of essential fatty acids (EFA). The specific requirements of EFAs in fish are dependent on their bioconversion and elongation capacity (Sargent et al., 2002). Freshwater fish generally require the dietary inclusion of polyunsaturated FAs (PUFA), while marine fish usually require the dietary inclusion of highly unsaturated FAs (HUFA) (Henry et al., 2015). However, a HUFA deficiency in the diet of marine fish may stimulate an adaptation mechanism by allowing a certain level of bioconversion of C18 PUFA to C20 or C22 HUFA (Henry et al., 2015; Oliva-Teles, 2012). The lipid level in FM (8.2%) is lower than that of insect meal, in which it ranges between 10 and 30%. Since the

lipid level and FA level and quality in insects is extremely variable, and their diet is the main responsible for these variations (Henry et al., 2015), it is possible to manipulate the lipid content and FA content and profile of insects feeding them with the desirable amounts of these components (Henry et al., 2015; St-Hilaire et al., 2007b).

The vitamin and mineral profiles of insects also depend on the composition of their diet. Nevertheless, the Ca and P levels are usually lower than that of FM, except for *Hermetia illucens* meal (Gasco et al., 2015; Henry et al., 2015).

In order to assess the use of insects in the fish diets, it is necessary to compare a control diet that covers all the fish requirements with an isonitrogenous and isoenergetic diet containing insect meal (Henry et al., 2015). These studies have been conducted with different fish species, using a wide range of different insects, at different stage (larvae, prepupae, pupae and adults) of different orders (Orthoptera, Isoptera, Coleoptera, Lepidoptera and Diptera) as FM replacement in aquaculture feeds. Some published results of these studies are summarized in **Appendix 1 and 2**.

European Union legislation concerning insects as food and feed

For putting a novel food and feed protein products on the market it should comply with European and national legislation for food and feed. Still, European law is not conclusive on several issues regarding the use of novel protein sources in feed and food products. **Table 1** shows an overview of the inconsistencies in EU legislation for insects as novel protein sources. In order to stimulate the use of novel protein sources in Europe, it is therefore important to adjust and clarify the European legislation (van der Spiegel et al., 2013).

According to Gasco et al. (2015), if the insect meal is considered as animal meal, it enters within the category of Processed Animal Protein (PAP), for which there was a prohibition of use, according to Reg. CE n. 999/2001, till 2013. However, recently the European Commission, with the Reg. CE n. 56/2013, re-authorised the use of PAPs obtained from non-ruminant farmed species in aquafeeds, except fishmeal which is already authorized, bringing some changes to the Annexes I and IV of Reg. CE n. 999/2001. However, before any modification of the Regulation, the member states require a global assessment of the possible hazards from the use of insects as PAPs in feeds for aquaculture species. For this reason, the European Commission already asked the official opinion of the EFSA (European Food Safety Authority) on the

microbiological, chemical and environmental hazards that may emerge from the production of insects as food and feed (Gasco et al., 2015).

Table 1. Overview of inconsistencies in EU legislation for insects as novel protein source (modified from van der Spiegel et al., 2013).

Protein source	Inconsistences in EU legislation	EU legislation
Insects	▪ Are insects and insect derived products novel foods?	▪ Reg. (EC) 258/97
	▪ Which feed/substrate can be used for insects?	▪ Reg. (EC) 767/2009; (EC) 1069/2009; (EU) 142/2011
	▪ Can food products consisting of/derived from insects be considered to be "products of animal origin"?	▪ Reg. (EC) 852/2004; (EC) 853/2004
	▪ Can insect proteins be used in feed of food-producing pigs and poultry?	▪ Reg. (EC) 999/2001; (EC) 142/2011; (EC) 1069/2009)

Black soldier fly (*Hermetia illucens*, L.)

Habitat and biology

Hermetia illucens (Linnaeus, 1758) is a Diptera fly that belongs to Stratiomyidae family, commonly known as black soldier fly (BSF), and is a coloniser of decaying material. This species is native from tropical, subtropical and temperate regions of America. The development of international transportation since the 1940s resulted in its dispersion and nowadays it is found in temperate and tropical regions throughout the world. The adult stage (**Fig. 5**) is black or blue and can achieve 15-20 mm in length while in the larval stage (**Fig. 6**) *H. illucens* is whitish colour and dull, with a small projecting head, and can achieve 27 mm in length and 6 mm in width (Diclaro and Kaufman, 2015; Harnden and Tomberlin, 2016).

The entire life cycle of *Hermetia illucens* lasts approximately 2 months, in ideal conditions. The female deposits a mass of about 500 eggs in dry fissures near or in decaying matter like organic wastes. In approximately 4 days larvae will hatch from the eggs and pass through six stages of development, during which they can feed quickly, from 25 to 500 mg of fresh matter/larva/day and a wide range of decaying organic matter. Before pupation, the sixth instar larvae (prepupae) disperse from the feeding place to dry sheltered areas to initiate pupation that require approximately 14 days but can be extremely variable and last up to 5 months. The females mate two days after emerging (Diclaro and Kaufman, 2015; Diener et al., 2011; Makkar et al., 2014).

Fig. 5. Adult BSF (*Hermetia illucens*. L.).

Source: INPN (U/D)

Fig. 6. Larvae of BSF (*Hermetia illucens*. L.).

Source: Jonathan Tan (U/D).

Nutritional attributes

Hermetia illucens larvae are considered a high-value feed source due its content in protein and fat. They contain approximately 40-44% of protein (dry matter basis) (DM) and its amino acid profile is particularly rich in lysine (6-8% of the protein). The amount of fat and fatty acids composition is largely dependent of the fatty acid composition of their diet (St-Hilaire et al., 2007b). According to this, it is possible to manipulate the lipid and fatty acid content of *H. illucens* larvae. Feeding them with a diet made of wastes containing desirable omega-3 fatty acids is, therefore, a way to enrich the final biomass (St-Hilaire et al., 2007b).

Ash content of *H. illucens* larvae is relatively high but variable, between 11 and 28% DM, and they are rich in calcium (5-8% DM) and phosphorus (0.6-1.5% DM) (St-Hilaire et al., 2007b; Tran et al., 2015). The dry matter content of fresh larvae is relatively high (35-45%), which makes them easier and less expensive to dehydrate than other fresh by-products (Newton et al., 2008).

Black soldier fly in fish diet

The effect of dietary inclusion of black soldier meal in fish diet is relatively limited. Nevertheless, the interest in this topic is increasing.

A study on the use of *H. illucens* in channel catfish (*Ictalurus punctatus*) and blue tilapia (*Oreochromis aureus*) diet showed that these species fed with larvae, alone or in combination with commercial diets (50% larvae and 50% commercial diet), had a similar performance (body weight and total length) in comparison with fish fed control diets. This study also showed that aroma and texture of channel catfish and blue tilapia fed larvae were also acceptable to the consumer (Bondari and Sheppard, 1981). Still, a later study (Bondari and Sheppard, 1987) had less positive results. Whereas larvae led to slower growth in sub-adult channel catfish grown in cages, the replacement did not reduce growth rate significantly if channel catfish were grown in culture tanks. On the other hand, replacement of 100% fishmeal by *H. illucens* did not allow good growth in channel catfish and blue tilapia reared in tanks, due to insufficient dry matter or crude protein. Zhang et al. (2014) demonstrated that 25% replacement of fishmeal by *H. illucens* larva powder produced no significant difference in the growth index and immunity index of yellow catfish when compared with those of the control group.

A feeding trial conducted by Kroeckel et al. (2012), was performed on juvenile turbot (*Psetta maxima*), using six diets containing defatted *H. illucens* meal in the following percentages of replacement of fishmeal 0, 17, 33, 49, 64, and 76%. Feed intake decreased with increasing *H. illucens* meal incorporation due to low palatability. Growth performance was high, but affected by dietary *H. illucens* meal inclusion. Specific growth rate was lower in all treatments containing insect meal, whereas feed conversion ratio was significantly higher at *H. illucens* meal inclusion levels superior than 33%. The presence of chitin might have influenced the feed intake, availability, and digestibility of the nutrients and therefore growth performance. Whole body protein content was not affected by treatment, while body lipid decreased with increasing insect meal inclusion levels in the diet (Kroeckel et al., 2012).

A study conducted by Lock et al. (2014) evaluated the effect of including *H. illucens* meal in the diet of Atlantic salmon (*Salmo salar*) in replacement of 25, 50 and 100% of fishmeal. Different nutrient isolation and processing techniques led to two insect meal types, A and B. The diets A25, A50 and A100 performed equally well as the control diet. The feed intake decreased moderately with increasing of insect meal inclusion but feed conversion ratio also decreased, resulting in an equal net growth of

the fish. No significant differences were detected between any of the dietary groups in histological analysis and sensory testing of fillets. The authors pointed out that the method of nutrient isolation and processing of the insect meal has an important impact on the performance of the product (Lock et al., 2014).

H. illucens larvae have been evaluated also as partial substitution of fishmeal in rainbow trout (*Oncorhynchus mykiss*), at 25 or 50% replacement level (St-Hilaire et al., 2007a). Results showed that 25% substitution had no adverse effect on the feed conversion ratio. Yet, for fish fed on diets containing 50% black soldier fly prepupae the total weight gain was lower (St-Hilaire et al., 2007a). In another experimental trial performed in rainbow trout, Sealey et al. (2011) used 25 or 50% replacement of fishmeal with *H. illucens* prepupae meal, conventional or enriched with fish offal. The growth of fish that fed enriched *H. illucens* diets was not significantly different from those of fish fed the fishmeal-based control diet, whereas the growth of fish fed normal *H. illucens* diets was significantly reduced compared with the fish fed the control diet (Sealey et al., 2011). A group of 30 untrained panellists did not detect any significant difference in a blind comparison among the feeding treatments (Sealey et al., 2011).

Aim of this study

The objectives of this study are to contribute for the development of more sustainable aquafeeds for *Oncorhynchus mykiss*, overcome the lack of information about quality of fish fed insect meal, and study the shelf life of fillets. Therefore, the effects of diets formulated to replace 25 or 50% of fishmeal by prepupae meal of *Hermetia illucens* on morphometric and marketable characteristics, physical and chemical characteristics of *Oncorhynchus mykiss* fillets were evaluated at different refrigerated times.

Materials and methods

The growth trial was carried out at the experimental facilities of the Department of Agricultural, Forest, and Food Sciences (DISAFA), University of Turin (Italy). The trial was directed by certified scientists (FELASA, category C), and all the procedures were conducted according to the recommendations of the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes.

Experimental diets

Experimental ingredient, i.e. *Hermetia illucens* (Black soldier flies, BSF) prepupae defatted meal (HI) (**Fig. 7**) was provided by Hermetia Deutschland GmbH & Co. KG (Germany). HI was obtained through rearing *Hermetia illucens* in small cages and controlling their holometabolic life cycle. Black soldier flies were fed bruised rye and wheat bran, according to European legislation (1069/2009 animal by products) that only allows the use of approved feeding stuff as substrate. *Hermetia illucens* prepupae were sacrificed by freeze at -20 °C for 48 hours and then were dried. The dried *Hermetia illucens* prepupae (90% dry mass) were defatted mechanically with high pressure.

Fig. 7. *Hermetia illucens* prepupae defatted meal.

Three experimental diets were formulated to be isoproteic (45% crude protein), isolipidic (15% crude lipids) and isoenergetic (22 MJ kg^{-1} gross energy). A control diet (HI0) was formulated and its main protein source was fish meal (FM). Two other diets were formulated similarly to the control diets but replacing FM by increasing levels of the experimental ingredient, HI, at 25% (HI25) or 50% (HI50) in feeds (dry matter basis). All the dietary ingredients were ground, well mixed and pelleted through 2.5 mm of diameter in a commercial meat grinder. After dried for 48 hours at 60 °C, pellets were screened and stored at -20 °C. The ingredients and the proximate composition of

the experimental diets, total amino acids in experimental diets, free amino acids in experimental diets and total fatty acids in experimental diets are presented in **Table 2**, **Table 3**and **Table 4**, respectively.

Table 2. Composition and proximate composition of experimental diets.

	Diet		
	HI0	**HI25**	**HI50**
Ingredient (g kg^{-1} DM)			
FM[1]	600	450	300
HI meal[2]	0	200	400
Wheat meal[3]	40	40	40
Wheat bran[4]	90	60	30
Starch gel, D500[5]	150	150	150
Fish oil[6]	90	70	50
Mineral mixture[7]	15	15	15
Vitamin mixture[8]	15	15	15
Proximate composition (% DM)			
Dry matter	96.36	96.48	95.88
Crude protein	41.10	39.84	40.25
Crude lipids	14.72	15.07	14.62
Ash	12.72	11.30	10.22
NDF[9]	3.27	3.42	7.19
ADF[10]	2.44	3.10	4.52
NFE[11]	30.11	31.48	31.24

[1] Fishmeal (90.4% DM, 66.7% crude protein, 8.3% crude lipids, 14.9% ash); Corpesca S.A., Santiago, Chile.
[2] *Hermetia illucens* prepupae defatted meal (93.09% DM, 52.82% crude protein, 22.49% crude lipids, 6.38% ash); Hermetia Deutschland GmbH & Co. KG, Germany.
[3] Wheat meal, Molino Spadoni S.P.A., Ravenna, Italy.
[4] Wheat bran, FA.MA.AR.CO S.P.A., Cuneo, Italy.
[5] Starch gel, UNIVAR S.P.A., Milan, Italy.
[6] Fish oil, GUGLIELMO PEARSON S.R.L., Genoa, Italy.
[7] Mineral mixture (g kg^{-1}): dicalcium phosphate, 500 g; calcium carbonate, 215 g; sodium salt, 40 g; potassium chloride, 90 g; magnesium chloride, 124 g; magnesium carbonate, 124 g; iron sulphate, 20 g; zinc sulphate, 4 g; copper sulphate, 3 g; potassium iodide, 4 g; cobalt sulphate, 0.02 g; manganese sulphate, 3 g; sodium fluoride, 1 g; Granda Zootecnici S.R.L., Cuneo, Italy.
[8] Vitamin mixture (IU or mg kg^{-1}): DL-α-tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; vitamin B_{12}, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2 mg; calcium panthotenate, 50 mg; choline chloride, 2000 mg; Granda Zootecnici S.R.L., Cuneo, Italy.
[9] Neutral detergent fibre, NDF (%) = [(weight in g at 105 °C) - (weight in g at 500 °C)]*100.
[10] Acid detergent fibre, ADF (%) = [(weight in g at 105 °C) - (weight in g of empty crucible)]*100.
[11] Nitrogen-free extract, NFE (%) = 100-%CP-%CL-%CF-%Ash.

Table 3. Total amino acids (AA) concentration (μmol mg^{-1} of dried and ground sample) in experimental diets.

		Diet		
		HI0	**HI25**	**HI50**
Essential AA	Arginine	0.002	0.003	0.003
	Histidine	0.001	0.002	0.002
	Isoleucine	0.001	0.002	0.002
	Leucine	0.003	0.004	0.004
	Lysine	0.002	0.003	0.002
	Methionine	0.001	0.000	0.000
	Phenylalanine	0.001	0.001	0.002
	Threonine	0.001	0.002	0.002
	Valine	0.002	0.003	0.003
Non-essential AA	Alanine	0.002	0.003	0.003
	Asparagine	0.002	0.003	0.003
	Cystine	0.000	0.000	0.000
	Glutamine	0.003	0.006	0.009
	Glycine	0.003	0.004	0.005
	Hydroxyproline	0.000	0.000	0.000
	Proline	0.002	0.007	0.012
	Serine	0.001	0.002	0.002
	Taurine	0.022	0.017	0.011
	Tyrosine	0.001	0.002	0.002

Table 4. Fatty acid content (as a percentage of total fatty acids) of experimental diets.

	Diet		
	HI0	**HI25**	**H50**
C12:0	3.30	14.43	24.41
C14:0	4.81	5.94	7.03
C16:0	12.80	12.74	12.53
C18:0	2.68	2.54	2.39
SFA	24.56	36.52	47.06
C16:1n7	5.97	5.51	5.27
C18:1n9	12.75	12.41	10.82
C18:1n7	3.03	2.43	1.85
C20:1n9	5.28	4.13	3.32
C22:1n11	4.28	3.37	2.78
MUFA	33.74	29.85	25.77
C18:2n6	3.12	4.20	4.79
PUFAn6	4.67	5.41	5.59
C18:3n3	1.05	1.13	1.02
C20:5n3	10.35	7.72	5.92
C22:5n3	1.50	1.28	0.98
C22:6n3	19.47	14.54	11.08
PUFAn3	35.62	27.16	20.87

The fatty acids SFA: C13:0, C15:0, C17:0, C20:0, C22:0, C24:0; MUFA: C14:1n5, C16:1n9, C17:1, C20:1n11, C20:1n7, C22:1n9, C22:1n7; PUFAn1 C16:4n1, C18:4n; PUFAn4: C16:2n4, C16:3n4, C18:2n4, C18:3n4; PUFAn6: C18:3n6 C20:2n6, C20:3n6, C20:4n6, C22:2n6, C22:4n6; PUFAn3, C20:3n3, C20:4n3, C21:5n3 were also detected, in percentage < 1%, but not reported in the table for brevity. They were anyway utilized to calculate the fatty acid groups and the total fatty acids.

Fish and rearing conditions

Rainbow trout (*Oncorhynchus mykiss*) juveniles were obtained from a local fish farm (Troticoltura Bessignana, Beinette, Cuneo, Italy) and transported, under appropriate conditions, to the experimental facilities of DISAFA, Unversity of Turin (Italy). Previously the growth trial starting, all fish were acclimatized to the outdoor rearing conditions during 3 weeks while fed a standard rainbow trout diet obtained from Skretting Italia SPA (Verona, Italy; www.skretting.it). After the adaptation period, a total of 360 specimens, with an initial body weight of 179 ± 10 g, were individually weighed and randomly distributed into 12 rectangular fiberglass tanks of 1000 L water capacity, supplied with an open and continuous freshwater system provided by artesian well water, allowing a water inflow of 8 L min^{-1} in each tank.

Diets were randomly assigned to quadruplicate groups of fish (initial body weight of 179 ± 10g) and fish were fed by hand to apparent visual satiation, twice a day (9:00 h and 15:00 h), six days a week (Monday to Saturday). In order to reduce the stress, in the first week the diet given to each tank was 1.2% of live weight. From the second week, the daily ration increased to 1.5% of the live weight of fish in the tanks. Extreme care was taken to assure that all feed supplied was consumed. Feed consumption was recorded daily and the water parameters (temperature, dissolved oxygen and pH) were measured every 15 days. Photoperiod was natural and the light conditions were similar for all the tanks. The water temperature was maintained at 13 ± 0.5 °C, dissolved oxygen (DO) at 8.9 ± 0.4 mg L^{-1} and pH ranged between 7.0 and 7.5. The trial lasted 78 days and during this period the feed intake was recorded every 15 days and the mortality daily.

Fish sampling

At the end of the feeding trial (78 days), all fish were individually weighed and n. 7 fish from each tank (n. 28 fish per treatment) were euthanized by an overdose of anaesthesia (tricaine methane-sulfonate (MS-222); 60 mg L^{-1}; PHARMAQ Ltd., United Kingdom). The day after slaughtering, the fish were transported, in refrigerated boxes, to the Laboratories of the Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), University of Florence (Italy), where morphological, marketable, and quality traits were analysed.

In order to investigate the effect of storage the fish were kept at refrigerated condition (1 °C) and analysed after 1, 3, 6 and 9 days of storage. During the storage, all fish were maintained in obscurity in polystyrene boxes, holed in the bottom, with ice covering. At each different *post mortem* time, the fish (n. 12 in total, i.e. n. 4 for each treatment) underwent to morphometric measurements and, subsequently the filleting, the right and left fillets were obtained from each fish. Right fillets were analysed as raw, while left fillets were analysed as cooked. Left fillets were weighed, vacuum packed in plastic bags and boiled (at 98-100 °C) for 5 minutes, until the core temperature arrived at 58 °C; then the bags containing the samples were removed from the water and cooled at room temperature and the fillets, removed from the bags, were weighed again. The details of the analyses performed on the whole fish and on the fillets (raw or cooked), are given below.

Morphometric and marketable characteristics

Fish were individually weighed and underwent to the following measurements using an orthometric meter: total and muscular body length (cm), head length (cm), and maximum height (cm). Then the fish were dissected and the liver, the muscle, and the viscera (whole viscera and separated liver) were separated and eviscerated body, right fillet, left fillet, skin of the right fillet, frame, fins and head were weighed.

From the linear and weight measures, morphometric characteristics, as dressed yield (DY), fillet yield (FY) (with and without skin), frame, fins and head percentages, condition factor (CF) and total wastes were calculated as follows:

DY (%) = [eviscerated weight (g) / body weight (g)] x 100

FY with skin (%) = [right fillet weight (g) + left fillet weight (g) / body weight (g)] x 100

FY without skin (%) = [(right fillet weight (g) – right skin weight (g)) x 2 / body weight (g)] x 100

Frame (%) = [frame weight (g) / body weight (g)] x 100

Fins (%) = [fins weight (g) / body weight (g)] x 100

Head (%) = [head weight (g) / body weight (g)] x 100

CF = [body weight (g) / total length^3 (cm)] x 100

Total wastes (%) = [frame + fins + head + viscera weight (g) / body weight (g)] x 100

Physical analyses

Colour, pH and texture

Colour measurements were performed in gills and both raw and cooked fillets, by a Spectrocolour®116 colorimeter (Bell Technology Ltd., Auckland, New Zealand) using the Spectral qc 3.6 software, according to the CIELab system (CIE, 1976). In this system, lightness (L*) is expressed in a scale ranging from 0 to 100%, i.e. 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). Chroma and Hue values were calculated as follows:

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}}$$

$$\text{Hue} = a^* \times \tan(b^*, a^*) \times 2$$

Colour was measured in triplicate on the epaxial, hypaxial, and caudal fillet regions and on the left gills. Finally, the colour of the fillet was expressed as mean of the values measured in the three sites.

Only in the raw fillets, the measurement of muscle pH was performed in triplicate on the cranial, medial and caudal fillet positions of the epaxial region, and the mean value for each fish was utilised in data analysis. For pH measurement, a Mettler Toledo DevenGo SG2™ pH-meter (Novate Milanese, Milano, Italy) equipped with an Inlab puncture electrode (Mettler-Toldedo, Ltd) was utilised.

For texture measurements, a Zwick Roell® 109 texturometer (Zwick Roell, Ulm, Germany), equipped with a 1 kN load cell and supplied by the Text Expert II software was utilized. The Warner-Bratzler (WB) shear test was performed on a muscle sample obtained from the cranial part of the epaxial region of the fillet (one measurement for each fillet). A straight blade (width of 7 cm), perpendicular to muscle fiber direction, was utilised at a crosshead speed of 30 mm/min to 50% of total deformation. Maximum shear force, defined as maximum resistance of the sample to shearing (Veland and Torrissen, 1999) was determined from the plot of force (N) compared with deformation (%). Texture measurements were performed in both raw and cooked fillets.

Water-holding capacity and cooking loss

Water Holding Capacity (WHC), performed only in the raw fillets, was determined by percentage of water loss after centrifugation, according to Eide et al. (1982) with the modification proposed by Hultmann and Rustad (2002). To carry out the analysis, fillet muscle was minced and 2 g were weighed in plastic tubes equipped with a filter net, that were centrifuged at 1500 rpm for 5 minutes. Finally, WHC was calculated as difference between the initial gross weight and the gross weight after centrifugation, and the value obtained was divided for the water content of the sample before centrifugation, determined by difference of the initial weight and after 24 hours at 105 °C. Two measurements for each sample were performed.

On the cooked fillets, the cooking loss (CL) was calculated by measuring the difference in weight of the fillet before the cooking process and after, according to the formula: CL (%) = [cooked sample weight (g) / raw sample weight (g)] x 100

After physical analyses, all fillets were minced. The individual ground fillets were frozen at −80 °C and freeze-dried at −18 °C (Heto PowerDry AC300-H freeze-dryer, Rodano, Italy). After freeze-dried, samples were quickly weighed and posteriorly they were exposed to air for 24 hours and weighed again. Finally, samples were crushed to a powder by a commercial grinder and collected in plastic bags that were stored in the dark at 4 °C until the moment of the analyses.

Chemical analyses

Proximate composition

Proximate composition of HI meal, of the three experimental diets, as well as of the freeze-dried fillets from the three groups of fish was determined according to AOAC (2000) procedures: dry matter (in oven at 105 °C to constant weight) was determined according to 950.46 method, ash (incinerated at 550 °C for 5 h) according to 920.153 method, crude protein (N x 6.25) by the Kjeldahl method after an acid digestion (Kjeltec 2300 Auto Analyzer, Tecator Höganas, Sweden) according to 976.05 method, and crude lipids extracted with petroleum ether in a Soxtec system (Extraction unit model 1043 and service unit model 1046, Tecator Systems, Höganäs, Sweden) according to 991.36 method. Proximate composition of fillets was determined in all samples (raw and cooked) analysed at the 1st and 9th post mortem days, to characterize the chemical composition of the fillets at the start (1st *post mortem* day) and at the end of the refrigerated (9th) storage. The crude fibre content was digested in sulphuric acid and sodium hydroxide solutions, and then the residue was calcinated. The difference in weight after calcination indicates the quantity of fibre in the sample. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed according to the procedure of Goering and Van Soest (1970).

Total lipid extraction

For the total lipid fraction of HI meal, of the experimental diets, as well as of raw and cooked fillets, the modified Folch et al. (1957) method was performed. A quantity of 2 g of each sample was homogenised by an Ultra-Turrax® (ULTRA-TURRAX model T25, Staufen, Germany) during 3 minutes with a chloroform-methanol (2:1 v/v) solution and then filtered. Distilled water with 0.88% KCl was added to the filtrate until the [Choloroform:Methanol:Water] ratio was 8:4:3, in order to obtain an effective extraction and separation. The tubes rested 24 hours to obtain a biphasic system. The lower phase, containing lipids dissolved in chloroform, was siphoned and recovered. After removal of the solvent (chloroform) by evaporation under vacuum and lipid resuspension in a known volume of chloroform (5 mL), 0.5 mL of lipid extract were utilised and the total lipid content was determined gravimetrically. The extracted lipids were used for the analysis of fatty acid profile

Fatty acid profile

For the fatty acid methyl ester (FAME) analysis of HI meal, of the experimental diets, as well as of fillets, the modified method of Morrison and Smith (1964) was performed. Lipids were saponified with 0.5 M KOH in methanol, and FAs were hydrolysed by adding 2 N HCl. Methyl esters were prepared by transmethylation, using boron fluoride-methanol at a 14% concentration. Methylated FAs were dissolved in petroleum ether, dried, and finally resuspended in 1 mL of hexane. The FAs composition was determined by gas chromatography (GC) using a Varian GC 430 gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector (FID); a Supelco Omegawax™ 320 capillary column (30 m x 0.32 mm i.d., 0.25 µm film and polyethylene glycol bonded phase; Supelco, Bellefonte, PA, USA) was utilised. The oven temperature was held at 100 °C for 2 minutes, increased to 160 °C over 4 minutes at the rate of 12 °C/min, and then increased 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. A quantity of 1 µL of sample in hexane was injected into the column with the carrier gas (helium) kept 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 computing integrator software

(Agilent). FAs were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco). Individual FAs were quantified using tricosanoic acid (C23:0) (Supelco) as internal standard. FAs were expressed as a percentage of total FAME. From the FAs profile, fat quality indexes as atherogenicity index (AI) and thrombogenicity index (TI), according to Ulbricht and Southgate (1991) and hypocholesterolemic/hypercholesterolemic FA ratio (HH), according to Santos-Silva et al. (2002) were calculated as follows:

AI (%) = [C12:0 + (4 × C14:0) + C16:0] / (Σn3 PUFA + Σn6 PUFA + ΣMUFA)

TI (%) = (C14:0 + C16:0 + C18:0) / [(0.5 × ΣMUFA) + (0.5 × Σn6 PUFA) + (3 × Σn3 PUFA) + (Σn3 PUFA / Σn6 PUFA)]

HH (%) = (C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3) / (C14:0 + C16:0)

Furthermore, PUFAn6/PUFAn6, PUFAn6/PUFAn3 and PUFA/SFA ratios were also calculated.

Lipid oxidation products

The content of conjugated dienes (CDs) and trienes (CTs) was measured by a colorimetric method, according to Srinivasan et al. (1996), 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 29 000 L mol^{-1} cm^{-1}. Results were expressed as mmol CD kg^{-1} sample. CTs were quantified at 268 nm (50 Scan spectrophotometer equipped with Cary Win UV software, Varian, Palo Alto, CA, USA) using a molar extinction coefficient of 29 000 L mol^{-1} cm^{-1}. Results were expressed as mmol CT kg^{-1} sample.

The 2-Thiobarbituric acid-reactive substances (TBARS) were measured by a colorimetric method at 532 nm, according to Salih et al. (1987). TBARS were extracted in 50 g L^{-1} thiobarbituric acid (TCA) and added with 0.04 mol L^{-1} tertiary butyl alcohol (TBA). After 40 minutes of incubation at 97 °C, the products were quantified with orientation to a calibration curve of 1,1,3,3-tetraethoxypropane (TEP) in 50 g L^{-1} TCA (0.8–8 µmol L^{-1}). Results were expressed as mg MDA (malondialdehyde) kg^{-1} sample.

Total amino acids

Total amino acids (TAA) extraction was performed according to a modified Potenza et al. (2013) method on each fillet sample, previously weighed (~ 1 mg) in a glass tube. Then, the experimental material was hydrolyzed in 200 µL 6 mol L^{-1} HCl solution at 120 °C for 24 h. After hydrolysis, 500 µL 0.1 mol L^{-1} HCl were added and the samples were submitted to the pre-column derivatization process. For each sample, 10 µL of solution was collected and placed in a glass vial 70 µL of solution of 0.0655 NaOH in AccQ•Tag borate buffer and 20 µL AccQ•Tag Ultra Reagent Powder (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, AQC). For each cycle of analysis, a calibration standard (CS) containing 17 proteinogenic amino acids, norvaline, taurine and hydroxyproline at a concentration of 10 pmol μL^{-1}, except cysteine, present at a concentration of 5 pmol μL^{-1}, was utilised. This solution was prepared with 40 µL 2.5 mmol L^{-1} amino acid standard solution, 20 µL 5 mmol L^{-1} Nval, 20 µL 5 mmol L^{-1} Tau and 20 µL 5 mmol L^{-1} Hypro in 880 µL Milli-Q water. CS (10 µL) was placed with 70 µL borate buffer and 20 µL AccQ•Tag Ultra Reagent Powder. All samples were mixed, heated to 55 °C for 10 minutes to finalize the derivatization reaction, and injected for UPLC analysis.

Free amino acids

For free amino acids (FAA) analysis, 100 mg from each sample (both insect meals, experimental diets and freeze-dry fish muscle) were homogenized in 5 mL of 0.5 M perchloric acid (PCA) using an ultrasound for 20 minutes and mixed in a heat bath (300 rpm at 60 °C for overnight). After centrifugation (4000 rpm at 25 °C for 10 minutes), 10 µL of cleared supernatant was transferred into glass vial insert. Lastly, samples were submitted to the pre-column derivatization process (as described for total amino acid determination) and injected for UPLC analysis.

UPLC conditions

AccQ•Tag Ultra derivatization kit (borate buffer and reagent), AccQ•Tag Ultra eluents A and B, HPLC-grade acetonitrile, and amino acid standard solution were

purchased from Waters (Miliford, MA, USA). Sodium hydroxide and constant-boiling hydrochloric acid solution was supplied by Merck (Darmstadt, Germany). Norvaline (Nval) was purchased from Sigma-Aldrich S.r.l. (Milan, Italy). Water was purified with Millipore, Milli-Q system (Massachusetts, USA). TAA and FAA composition were determined by a Waters AcquityTM Ultra Performance Liquid Chromatography (UPLCTM) system (Waters, Miliford, MA, USA) equipped with an AcquityTM tunable ultraviolet (TUV) detector (detection wavelength 260 nm) and Acquity UPLCTM column (BEH C18, 2.1 x 100 mm, 1.7 µm particle size). Two different eluents were used: A (AccQ•Tag Ultra eluent A, diluted in the ratio 1:20 with Milli-Q water) and B (AccQ•Tag Ultra eluent B). The gradient eluent program was: 0.54 min, 0.1% B; 5.74 min, 9.1% B; 8.73 min, 0.1% B; 9.50 min, 0.1% B. The column temperature was 55 °C, the flow-rate was of 0.7 mL min^{-1}, and the injection volume was 1 µL (partial loop). Data were collected and analysed by the Waters Empower™ chromatography software version 2.0.

Statistical analyses

Data were analysed by two-way ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The factors diet (3 levels: HI0, HI25, HI50) and time of storage (4 levels: T1, T3, T6, T9) and their interaction were considered in order to understand if they have a significant effect on the evaluated parameters. Differences among means were separated using Tukey's test (SAS Inst. Inc.) at $p < 0.05$. Body weight was considered as covariate in order to prevent that the effect of diet and time of storage on evaluated parameters has been hidden by the effect of this factor.

Results

Morphometric and marketable characteristics

All experimental diets were immediately accepted by fish and no mortality occurred during the trial. Morphometric parameters and marketable yields are presented in **Table 5** as mean values of individuals grouped by diet, regardless of the storage time. Furthermore, effects of diet, time of storage and body weight are shown. Only body weight was significantly affected by diet. Individuals fed by HI25 had the highest body weight, while individuals fed by HI50 had a body weight higher in value than the control ones (HI0), but not statistically different of both HI0 and HI25. No more variables were significantly affected by diet, time of storage, their interaction and body weight.

Table 5. Morphometric and commodity-related parameters of fish fed the experimental diets.

	Diet (D)			Significance				RSD[1]
	HI0	**HI25**	**HI50**	**D**	**T[2]**	**DxT**	**W[3]**	
Body weight, g	557.494[b]	646.90[a]	593.97[ab]	*	ns	ns	-	97.66
Total length, cm	37.48	38.69	38.21	ns	ns	ns	ns	1.83
Muscle length, cm	34.58	35.80	35.39	ns	ns	ns	ns	1.79
Frame, %	9.75	8.45	9.53	ns	ns	ns	ns	2.30
Fins, %	1.24	1.08	1.25	ns	ns	ns	ns	0.27
Head, %	14.11	13.75	13.69	ns	ns	ns	ns	1.10
Total wastes, %	25.10	23.30	24.47	ns	ns	ns	ns	2.60
Dressed yield, %	88.01	86.06	88.38	ns	ns	ns	ns	4.51
Fillet yield with skin, %	61.71	63.57	62.97	ns	ns	ns	ns	2.74
Fillet yield without skin, %	53.20	55.50	55.05	ns	ns	ns	ns	3.75
Condition factor, g/cm^3	1.05	1.11	1.06	ns	ns	ns	ns	0.08

Means in the same row with different superscript letters are significantly different; ns: not significant; *: $p < 0.05$;
[1]Residual Standard Deviation; [2]Time; [3]Body weight.

Physical analyses

Colour analyses

The colour values of the left gill as mean values of individuals grouped by diet and by time of storage are presented in **Table 6**. The effect of the diet, time, their interaction and effect of body weight are also expressed in the same table. Left gills of fish fed different diets presented no differences in colour. In contrast, the effect of time was significant for all the parameters that express the colour characteristics, as expected, since the changes in colour during the storage are known for skin, gill and muscle. The value of L*, which represents lightness, and the value of Hue, which represents how we perceive colour, decreased from T1 to T3 but remained stable after this time, with no significant differences among T3, T6 and T9. In contrast, the redness index (a*), the yellowness index (b*) and Chroma, which represents colour saturation, increased in the first period of storage (from T1 to T3) and remained stable for the rest of the time, with no significant differences among T3, T6 and T9. Effect of the interaction diet × time was not significant, indicating that individuals fed different diet presented a comparable evolution of colour characteristics during the refrigerated storage. Furthermore, left gills colour was not significantly affected by body weight.

Table 6. Colour values of the left gill of fish fed the experimental diets, at different storage time.

	Diet (D)			Time (T) *				Significance				RSD[1]
Colour	**HI0**	**HI25**	**HI50**	**T1**	**T3**	**T6**	**T9**	**D**	**T**	**D×T**	**W[2]**	
L*[3]	19.33	18.47	18.89	43.90^{a}	11.89^{b}	6.91^{b}	12.88^{b}	ns	***	ns	ns	8.12
a*[4]	21.52	23.73	23.05	2.74^{b}	32.55^{a}	28.77^{a}	27.01^{a}	ns	***	ns	ns	9.66
b*[5]	9.79	10.28	10.40	4.00^{b}	13.78^{a}	11.01^{a}	11.84^{a}	ns	**	ns	ns	5.35
Chroma[6]	24.51	26.57	25.84	6.52^{b}	35.52^{a}	30.87^{a}	29.65^{a}	ns	***	ns	ns	10.67
Hue[7]	33.99	36.54	33.29	73.58^{a}	21.98^{b}	19.24^{b}	23.60^{b}	ns	***	ns	ns	8.05

Means in the same row with different superscript letters are significantly different; ns: not significant; **: $p < 0.01$; ***: $p < 0.001$.

* As interaction (DxT) was not significant, each value represents the means for the three diets.

[1]Residual Standard Deviation; [2]Body weight; [3]Lightness; [4]Redness; [5]Yellowness; [6]Saturation; [7]Perception of colour.

In **Table 7**, the colour values of muscle of the raw and cooked fillet are presented as mean values of the individuals grouped by diet and by time of storage. The effect of the diet, time, their interaction and the effect of body weight are also expressed. As in the left gills, the diet individually and their interaction with time had no

significant effect on colour values of muscle. On the raw fillet, time individually had a significant effect ($p < 0.001$) on L*, which followed the same decreasing trend as in the gills. On the cooked fillet, instead, time had a significant effect on L*, b* and Chroma values. For all these three indexes, the cooked samples showed higher values at T3 in comparison with samples analysed at the other storage times. The body weight had in its place a significant effect ($p < 0.05$) on a* and Hue values of the raw fillet and on Hue value of the cooked one. Still, for both fillet no significant differences were observed among different times of storage. Increased of all values in cooked compared to raw fillets was observed.

Table 7. Colour values of raw and cooked dorsal fillets of fish fed the experimental diets in different storage time.

	Diet (D)			Time (T)				Significance				RSD[1]
Colour	**HI0**	**HI25**	**HI50**	**T1**	**T3**	**T6**	**T9**	**D**	**T**	**D×T**	**W[2]**	
Raw fillet												
L*[3]	33.98	33.29	34.14	45.94^{a}	30.28^{b}	29.78^{b}	29.22^{b}	ns	***	ns	ns	3.13
a*[4]	-0.25	-0.71	0.04	-0.13	-0.05	0.83	0.21	ns	ns	ns	*	1.41
b*[5]	4.18	4.05	4.26	3.68	5.24	3.63	4.11	ns	ns	ns	ns	1.67
Chroma[6]	4.41	4.32	5.01	4.23	5.65	3.99	4.45	ns	ns	ns	ns	1.78
Hue[7]	97.00	109.01	103.20	100.15	96.16	111.47	104.50	ns	ns	ns	*	17.80
Cooked fillet												
L*	76.17	74.66	76.46	75.30^{b}	80.71^{a}	74.89^{b}	72.15^{b}	ns	**	ns	ns	4.26
a*	-0.44	0.82	1.06	-0.88	-0.61	-0.97	-0.64	ns	ns	ns	ns	0.69
b*	12.59	13.35	13.92	11.94^{b}	15.29^{a}	12.19^{b}	13.73^{ab}	ns	**	ns	ns	2.32
Chroma	12.65	13.44	14.03	12.04^{b}	15.34^{a}	12.31^{b}	13.79^{ab}	ns	**	ns	ns	2.26
Hue	92.79	95.12	95.79	95.53	92.74	96.50	93.50	ns	ns	ns	*	4.18

Means in the same row with different superscript letters are significantly different; ns: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

* As interaction (DxT) was not significant, each value represents the means for the three diets.

[1]Residual Standard Deviation; [2]Body weight; [3]Lightness; [4]Redness; [5]Yellowness; [6]Saturation; [7]Perception of colour.

pH, texture, water-holding capacity and cooking loss

Data regarding pH, texture in raw fillets, texture in cooked fillets, WHC and CL are shown in **Figures 8**, **9**, **10**, **11** and **12**, respectively, as mean values of storage of the three groups differently fed by control diet (HI0) and experimental diets (HI25 and

HI50), at each time. The time of storage had a significant effect ($p < 0.05$) on almost all variables measured.

Concerning the pH values (**Fig. 8**), as aforementioned, the time of storage had a significant effect ($p < 0.05$) on it, where mean value was 6.37 (± 0.053) at T1 and increased to 6.46 (± 0.054) at T3, posteriorly decreased to 6.44 (± 0.048) at T6 (± 0.058) and increased again to 6.46 at T9. No significant effect of diet on pH was observed. The shear force of the raw fillets was not significantly affected by the diet (**Fig. 9**). The temporal evolution presented mean values of 19.42 N (± 3.172) at T1, 11.35 N (± 3.577) at T3, 9.37 N (± 2.342) at T6, and 13.47 N (± 2.162) at T9. As in raw fillets, the shear force of the cooked fillets (**Fig. 10**) was not significantly affected by the diet. Mean values underwent a similar evolution, 9.89 N (± 3.846) at T1, 12.34 N (± 4.713) at T3, 6.768 N (± 1.569) at T6 and 9.173 N (± 3.751) at T9.

The WHC values (**Fig. 11**), as aforementioned, were significantly affected ($p < 0.05$) by the time of storage. The mean value at T1 was 86.96% (± 2.946) and increased to 90.80% at T3 (± 1.173), posteriorly decreased to 87.97% (± 2.270) at T6 and increased again to 90.52% at T9 (± 2.536), demonstrating the same tendency of pH. No significant effect of diet on WHC was observed.

Regarding CL (**Fig. 12**), the effect of diet on all samples, irrespective of the storage time, was significant ($p < 0.05$). Individuals fed by H50 had the highest values of CL, followed by HI25 (without significantly difference from the other diets) and finally by HI0. The mean value of HI0 along the storage time was 7.45 (± 1.683), while the mean value of H25 was 9.25 (± 2.230) and the mean value of HI50 was 10.99 (± 4.245). Contrarily to pH, shear force and WHC, no significant effect of storage time on CL was observed.

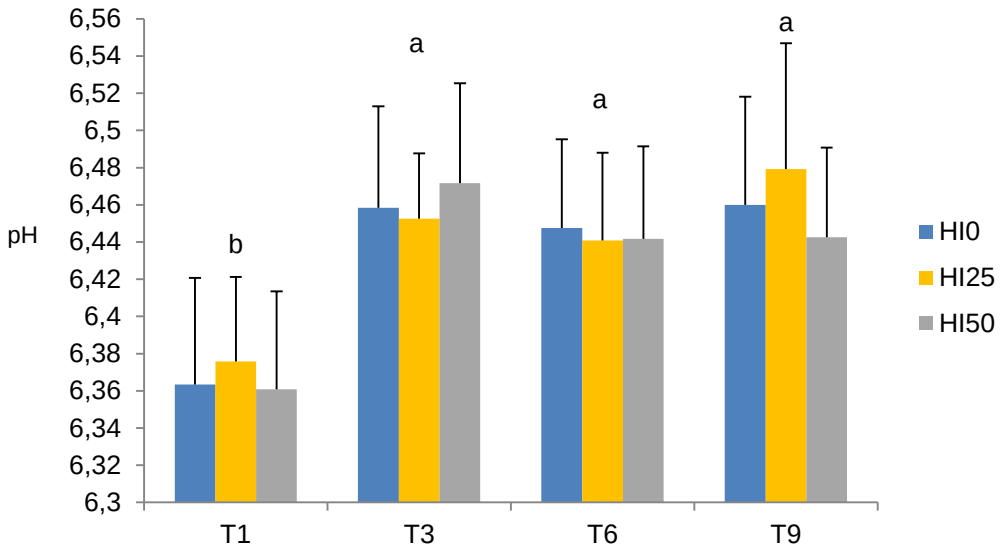

Fig. 8. pH values of the raw fillets of fish fed experimental diets, at each time of storage. Storage times with different letters are significantly different ($p < 0.05$). No significant effect of diet was observed.

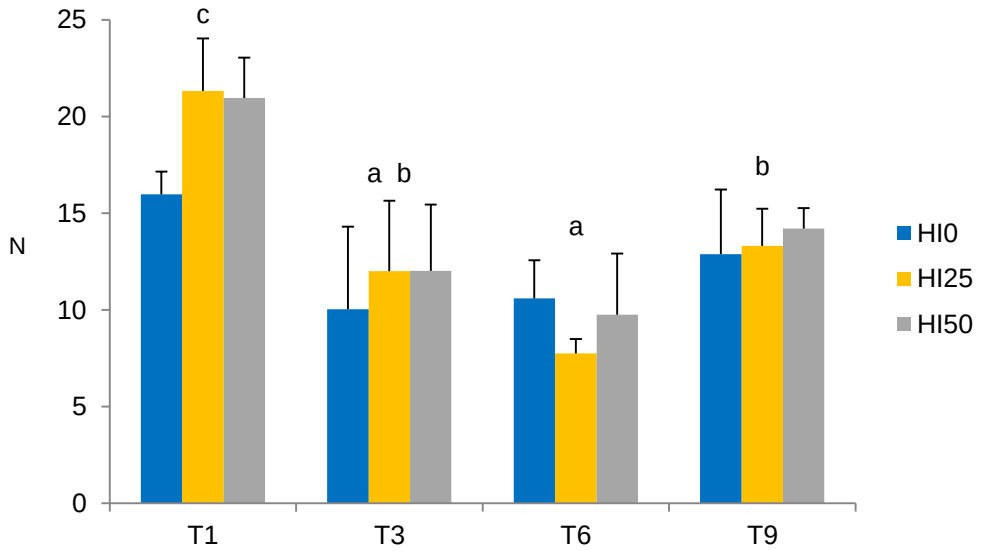

Fig. 9. Shear force (N) of raw fillets: mean values of raw fillets of the fish fed experimental diets, at each time of storage. Storage times with different letters are significantly different ($p < 0.05$). No significant effect of diet was observed.

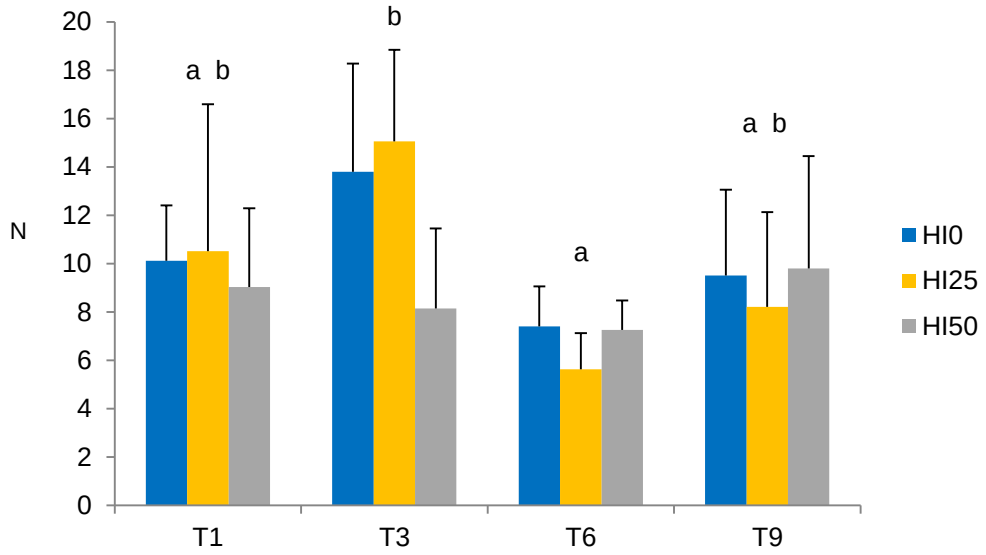

Fig. 10. Shear force (N) of cooked fillets: mean values of cooked fillets of the fish fed experimental diets, at each time of storage. Storage times with different letters are significantly different ($p < 0.05$). No significant effect of diet was observed.

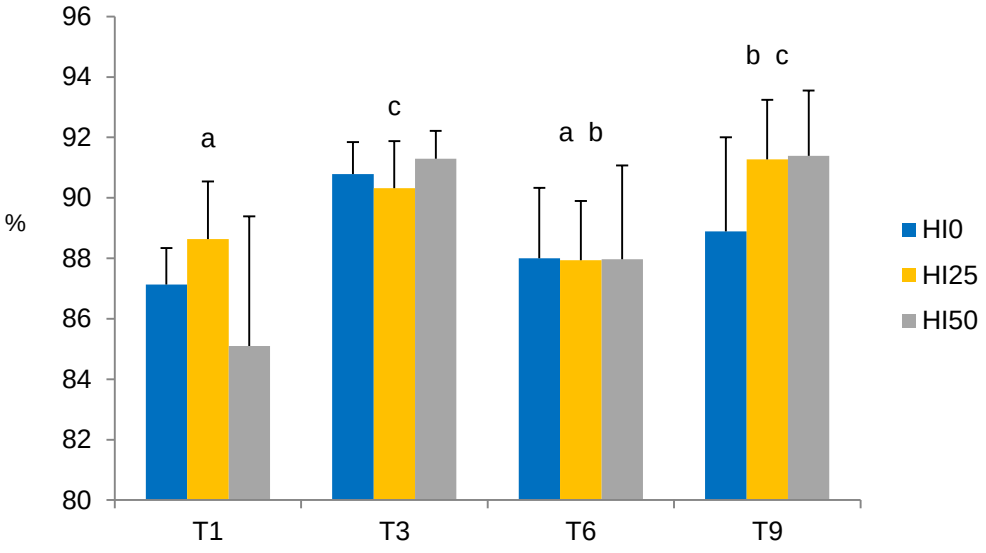

Fig. 11. WHC values (%) of the raw fillets of fish fed experimental diets, at each time of storage. Storage times with different letters are significantly different ($p < 0.05$). No significant effect of diet was observed.

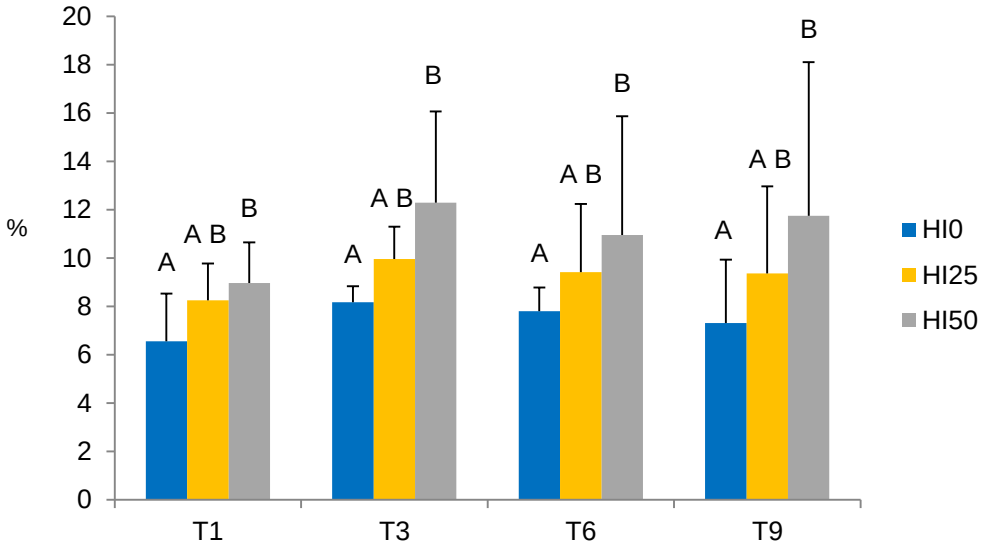

Fig. 12. CL values (%) of the cooked fillets of fish fed experimental diets, at each time of storage. Experimental diets with different letters are significantly different ($p < 0.05$). No significant effect of storage time was observed.

Chemical Analyses

Proximate composition

In **Table 8** are presented the results of proximate analyses of both raw and cooked fillets as mean values of individuals grouped by diet and by time of storage. The effect of the diet, time, their interaction and the effect of body weight are also expressed in the same table. No significant effect of diet on both raw and cooked fillets proximate composition was observed and significant effect of body weight was observed only in total lipids of cooked fillets, whereas interaction between diet and time had a significant effect only on the crude protein (CP) and ether extract (EE) content of raw fillets. In contrast, time had a significant effect on all parameters of raw fillets, excluding only CP. Lipid content in particular (both as ether extract in fresh fillet and as total lipids in fresh and cooked fillet) showed a decreased from the start of storage period (T1) to the end of this period (T9), while moisture and ash increased in fresh fillet.

Table 8. Proximate composition (%) of raw and cooked fillets of fish fed the experimental diets in different storage time.

	Diet (D)			Time (T)		Significance				RSD[1]
	HI0	**HI25**	**HI50**	**T1**	**T9**	**D**	**T**	**D×T**	**W[2]**	
Raw fillet										
Water	71.92	71.55	70.84	70.43[b]	72.44[a]	ns	**	ns	ns	1.41
Crude protein	19.11	19.47	19.45	19.34	19.34	ns	ns	*	ns	0.41
Ether extract	7.66	7.48	8.29	8.77[a]	6.85[b]	ns	**	*	ns	1.31
Ash	1.32	1.36	1.33	1.29[b]	1.39[a]	ns	**	ns	ns	0.07
Total lipids	8.05	8.68	9.11	11.64[a]	7.12[b]	ns	***	ns	ns	1.46
Cooked fillet										
Water	71.39	71.20	71.22	71.08	71.44	ns	ns	ns	ns	1.56
Crude protein	20.85	20.95	20.83	20.97	20.78	ns	ns	ns	ns	0.65
Ether extract	6.29	6.63	6.54	6.54	6.43	ns	ns	ns	ns	1.35
Ash	1.37	1.28	1.28	1.33	1.28	ns	ns	ns	ns	0.15
Total lipids	7.16	7.29	7.48	8.44[a]	6.69 [b]	ns	**	ns	**	1.23

Means in the same row with different superscript letters are significantly different; ns: not significant; *: $p < 0.05$; **: $p < 0.01$.
[1]Residual Standard Deviation; [2]Body weight.

Fatty acids profile

Data regarding the fatty acid profile of the raw fillets are presented in **Table 9**. Fatty acid profile was significantly affected ($p < 0.001$) by diet. In general, SFA percentage increased with the increasing levels of inclusion of *Hermetia illucens* prepupae meal in the diet, whereas MUFA and PUFA incidences decreased. Concerning to SFA, the percentage of lauric acid (C12:0) suffered the major modifications, with fillets of individuals fed by HI50 having the highest percentage of this FA, while individuals fed by HI0 had the lower. The amount of stearic acid (C18:0) was instead not affected by the diet. MUFA percentage, in contrast, decreased with the increasing levels of inclusion of *Hermetia illucens* prepupae in the diet. Single fatty acids that were significantly affected were C18:1n7 (vaccenic acid), C20:1n9 (gadoleic acid) and C22:1n11 (eicosenoic acid). The amount of C16:1n7 (palmitoleic acid) and 18:1n9 (oleic acid) were not affected by the diet. Concerning to PUFA of the n3 series, it was observed a decrease in the incidence of this group of fatty acids along with the crescent inclusion of *Hermetia illucens* prepupae in the diet. The most outstanding differences were found for C20:5n3 (eicosapentaenoic acid, EPA) and C22:6n3

(docosahexaenoic acid, DHA). The group of PUFA of the n6 series was the only one that was not affected by the changes in the diet.

The time of storage had a significant effect only on C18:0 percentage ($p < 0.01$) and C16:1n7 ($p < 0.05$), but no specific trend of decrease or increase was evident. The effect of the interaction between diet and time of storage was not significant, except for C18:0. It has to be noticed also that body weight significantly affected the percentages of the most of the fatty acids.

Table 9. Fatty acid (in % of total fatty acids) profile of raw fillets of fish fed the experimental diets, at different storage time.

	Diet (D)			Time (T)				Significance				RSD[1]
Fatty acid	**HI0**	**HI25**	**HI50**	**T1**	**T3**	**T6**	**T9**	**D**	**T**	**D×T**	**W[2]**	
C12:0	0.59^{c}	6.08^{b}	11.80^{a}	5.80	6.21	6.36	6.25	***	ns	ns	**	0.54
C14:0	3.03^{c}	4.12^{b}	5.27^{a}	4.15	4.21	3.99	4.21	***	ns	ns	**	0.44
C16:0	15.01^{b}	15.11^{b}	15.34^{a}	15.14	15.12	15.28	15.06	**	ns	ns	***	0.29
C18:0	3.59	3.57	3.56	3.68^{a}	3.47^{c}	3.58^{b}	3.58^{b}	ns	**	**	***	0.13
SFA	23.07^{c}	29.63^{b}	36.61^{a}	29.52	29.74	29.97	29.86	***	ns	ns	***	0.79
C16:1n7	5.22	5.32	5.48	5.29^{a}	5.52^{a}	5.39^{a}	5.15^{ab}	ns	*	ns	ns	0.32
18:1n9	22.01	21.74	21.53	22.15	21.69	21.65	21.56	ns	ns	ns	**	0.69
C18:1n7	2.78^{a}	2.52^{b}	2.29^{c}	2.55	2.53	2.53	2.50	***	ns	ns	ns	0.07
C20:1n9	2.50^{a}	2.19^{b}	1.87^{c}	2.22	2.16	2.18	2.16	***	ns	ns	**	0.13
C22:1n11	1.40^{a}	1.03^{b}	0.73^{c}	1.07	1.02	1.08	1.05	***	ns	ns	*	0.11
MUFA	35.40^{a}	34.13^{b}	33.19^{c}	34.67	34.30	34.21	33.79	***	ns	ns	ns	0.99
C18:2n6	7.74	7.90	7.83	7.98	7.63	7.84	7.85	ns	ns	ns	***	0.47
PUFAn6	10.16	10.06	9.96	10.19	9.91	10.03	10.11	ns	ns	ns	***	0.50
C18:3n3	1.86^{a}	1.73^{b}	1.51^{c}	1.73	1.66	1.72	1.69	***	ns	ns	***	0.10
C20:5n3	4.35^{a}	3.33^{b}	2.25^{c}	3.27	3.29	3.36	3.33	***	ns	ns	ns	0.22
C22:5n3	2.49^{a}	1.87^{b}	1.24^{c}	1.91	1.91	1.81	1.84	***	ns	ns	ns	0.15
C22:6n3	19.47^{a}	16.62^{b}	13.30^{c}	16.15	16.63	16.21	16.86	***	ns	ns	ns	0.94
PUFAn3	30.28^{a}	25.21^{b}	19.51^{c}	24.70	25.15	24.82	25.33	***	ns	ns	ns	1.04

Means in the same row with different superscript letters are significantly different; ns: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

[1]Residual Standard Deviation; [2] Body weight.

The fatty acid profile of the cooked fillets, presented in **Table 10**, was very similar to the profile of the raw fillets in the amount of each fatty acid and trends. Nevertheless, PUFA of n3 series slightly increased to the detriment of MUFA. Regarding to SFA, particularly lauric acid, increased along with the crescent inclusion of *Hermetia illucens* prepupae in the diet, while MUFA and PUFA of n3 series (EPA and DHA, in particular) were contained in greater incidence in fish fed the control diet. Storage time and their interaction with diet had a significant effect only on C18:0 ($p < 0.05$ and $p < 0.01$, respectively), meaning that C18:0 percentage was affected by time of storage and that the three experimental diets followed a different progression. Also for the cooked fillets, body weight significantly affected the percentages of the most of the fatty acids.

Quality indexes were calculated from fatty acids profile, with the aim to characterize the nutritional/functional properties of the fillets. The values for Atherogenicity Index (AI), Thrombogenicity Index (TI), Hypocholesterolemic /Hypercholesterolemic FA ratio (HH) and PUFAn3/PUFAn6 ratio were calculated for raw and cooked fillets and the results are presented in **Table 11**. Diet had a significant effect ($p < 0.001$) on all indexes of both raw and cooked fillet. AI, TI and PUFAn6/PUFAn3 ratio tended to increase with the crescent inclusion of *Hermetia illucens* prepupae meal in the diet. In contrast, HH, PUFAn3/PUFAn6 and PUFA/SFA ratio are higher in fish fed the control diet and progressively lower. The effect of storage time was significant ($p < 0.01$) only on the TI index of raw fillet, as it slightly decreased from T1 to T3, but it remained stable from T3 to T9. However, significant effect of interaction between time of storage and diet was not reported. Instead, all indexes were significantly affected by body weight, except TI of the raw fillet. Comparing raw and cooked fillet, small differences are present; with the cooking process the PUFAn3/PUFAn6 and PUFA/SFA ratio increased.

Table 10. Fatty acid (in % of total fatty acids) profile of cooked fillets of fish fed the experimental diets, at different storage time.

	Diet (D)			Time (T)				Significance				RSD[1]
Fatty acid	**HI0**	**HI25**	**HI50**	**T1**	**T3**	**T6**	**T9**	**D**	**T**	**D×T**	**W[1]**	
C12:0	0.52^{c}	5.76^{b}	11.44^{a}	5.65	5.89	5.87	6.21	***	ns	ns	**	0.70
C14:0	3.11^{c}	4.06^{b}	4.85^{a}	3.60	4.16	4.12	4.14	***	ns	ns	ns	0.75
C16:0	15.02^{b}	15.11^{b}	15.44^{a}	15.18	15.30	15.29	14.98	**	ns	ns	***	0.35
C18:0	3.58	3.60	3.60	3.69^{a}	3.51^{b}	3.59^{ab}	3.58^{ab}	ns	*	**	**	0.13
SFA	23.05^{c}	29.25^{b}	35.98^{a}	28.84	29.59	29.60	29.68	***	ns	ns	***	0.97
C16:1n7	5.07	5.12	5.31	5.06	5.35	5.20	5.06	ns	ns	ns	ns	0.31
C18:1n9	21.33	21.13	20.91	21.43	21.00	20.90	21.17	ns	ns	ns	ns	0.68
C18:1n7	2.74^{a}	2.48^{b}	2.25^{c}	2.49	2.51	2.48	2.47	***	ns	ns	ns	0.07
C20:1n9	2.45^{a}	2.16b	1.83^{c}	2.17	2.13	2.14	2.15	***	ns	ns	*	0.14
C22:1n11	1.38^{a}	1.04^{b}	0.72^{c}	1.04	1.03	1.07	1.06	***	ns	ns	ns	0.12
MUFA	34.44^{a}	33.26^{b}	32.30^{c}	33.54	33.38	33.16	33.26	***	ns	ns	ns	0.97
C18:2n6	7.45	7.61	7.65	7.76	7.29	7.55	7.68	ns	ns	ns	***	0.48
PUFAn6	9.90	9.87	9.87	10.11	9.64	9.80	9.98	ns	ns	ns	***	0.54
C18:3n3	1.82^{a}	1.68^{b}	1.48^{c}	1.69	1.60	1.68	1.67	***	ns	ns	***	0.10
C20:5n3	4.46^{a}	3.41^{b}	2.35^{c}	3.37	3.39	3.49	3.38	***	ns	ns	ns	0.26
C22:5n3	2.54^{a}	1.89^{b}	1.31^{c}	1.98	1.91	1.87	1.89	***	ns	ns	ns	0.15
C22:6n3	20.68^{a}	18.07^{b}	14.82^{c}	18.04	18.01	17.78	17.59	***	ns	ns	ns	1.00
PUFAn3	31.57^{a}	26.69^{b}	21.15^{c}	26.65	26.50	26.56	26.18	***	ns	ns	*	1.11

Means in the same row with different superscript letters are significantly different; ns: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
[1]Residual Standard Deviation; [2] Body weight.

Table 11. Nutritional indexes of raw and cooked fillets of fish fed the experimental diets, at different storage time.

	Diet (D)			Time (T)				Significance				RSD[2]
	HI0	**HI25**	**HI50**	**T1**	**T3**	**T6**	**T9**	**D**	**T**	**D×T**	**W[2]**	
Raw Fillet												
AI	0.37^{c}	0.54^{b}	0.77^{a}	0.55	0.57	0.56	0.57	***	ns	ns	***	0.03
TI	0.13^{c}	0.17^{b}	0.23^{a}	0.19^{a}	0.17^{b}	0.17^{b}	0.17^{b}	***	**	ns	Ns	0.01
HH	3.14^{a}	2.72^{b}	2.29^{c}	2.71	2.70	2.71	2.73	***	ns	ns	***	0.13
n3/n6	3.00^{a}	2.52^{b}	1.97^{c}	2.45	2.54	2.49	2.51	***	ns	ns	***	0.17
n6/n3	0.34^{c}	0.40^{b}	0.51^{a}	0.42	0.41	0.42	0.41	***	ns	ns	***	0.02
PUFA /SFA	1.82^{a}	1.23^{b}	0.82^{c}	1.28	1.30	1.29	1.31	***	ns	ns	***	0.09
Cooked Fillet												
AI	0.37^{c}	0.53^{b}	0.73^{a}	0.51	0.56	0.55	0.56	***	ns	ns	**	0.05
TI	0.13^{c}	0.15^{b}	0.20^{a}	0.16	0.16	0.16	0.16	***	ns	ns	**	0.01
HH	3.14^{a}	2.76^{b}	2.38^{c}	2.85	2.71	2.71	2.77	***	ns	ns	***	0.16
n3/n6	3.20^{a}	2.72^{b}	2.15^{c}	2.66	2.76	2.73	2.63	***	ns	ns	***	0.18
n6/n3	0.31^{c}	0.37^{b}	0.47^{a}	0.39	0.38	0.38	0.39	***	ns	ns	***	0.02
PUFA/SFA	1.87^{a}	1.29^{b}	0.88^{c}	1.37	1.34	1.33	1.35	***	ns	ns	***	0.08

Means in the same row with different superscript letters are significantly different; ns: not significant; ***: $p < 0.001$.; [1]Residual Standard Deviation; [2]Body weight.

Lipid oxidation products

Data regarding CD, TD and TBARS are shown in **Fig. 13**, **14** and **15** respectively, as mean values at each time of storage of the three groups fed by control diet (HI0) or experimental diets (HI25 and HI50). Concerning to CD levels of fillets (**Fig. 13**) and despite no significant effect of experimental diets on CD was observed, at T1 the mean values decreased with greater content of *Hermetia illucens* prepupae in the diets. In contrast, at T9 the mean values decreased between HI0 and HI25, but increased again between HI25 and HI50. Furthermore, the CD levels of fillets at T9 were higher than T1, irrespective of experimental diet. Regarding to CT mean values of fillets (**Fig. 14**), the same trend described for CD levels was observed, except in fillets of fish fed the HI25, where the CT mean values decreased from T1 to T9. Nonetheless, significant effect of experimental diets on CD was observed, where the mean values of HI0 were significantly different from other experimental diets. Concerning the formation of oxidation by-products measured by TBARS level in fillets (**Fig. 15**), the mean values decreased with the higher content of *Hermetia illucens* prepupae in the diets, irrespective of the storage time. In contrast, from T1 to T9 the mean values of oxidation by-products increased, irrespective of experimental diet.

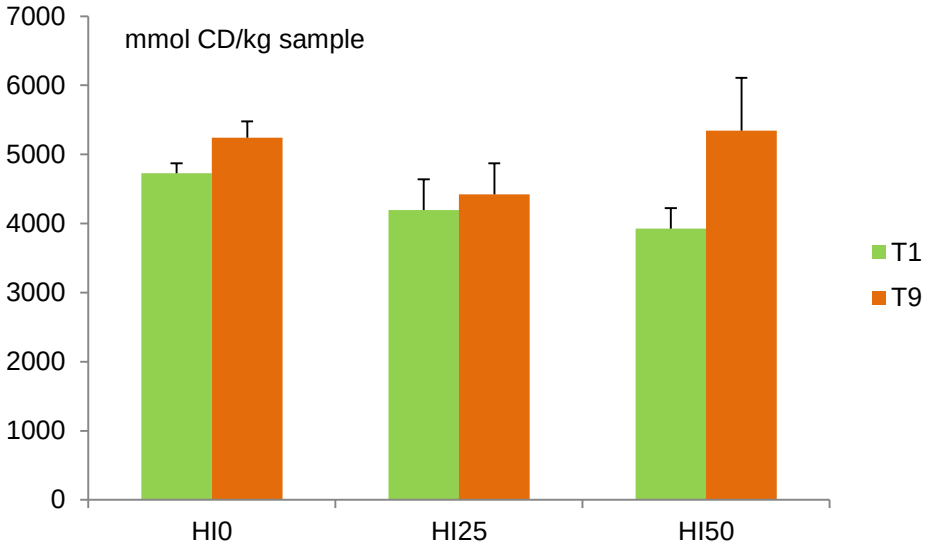

Fig. 13. CD values (mmol CD/kg sample) of fish fed experimental diets, at different times of storage. No significant effect of experimental diets was observed.

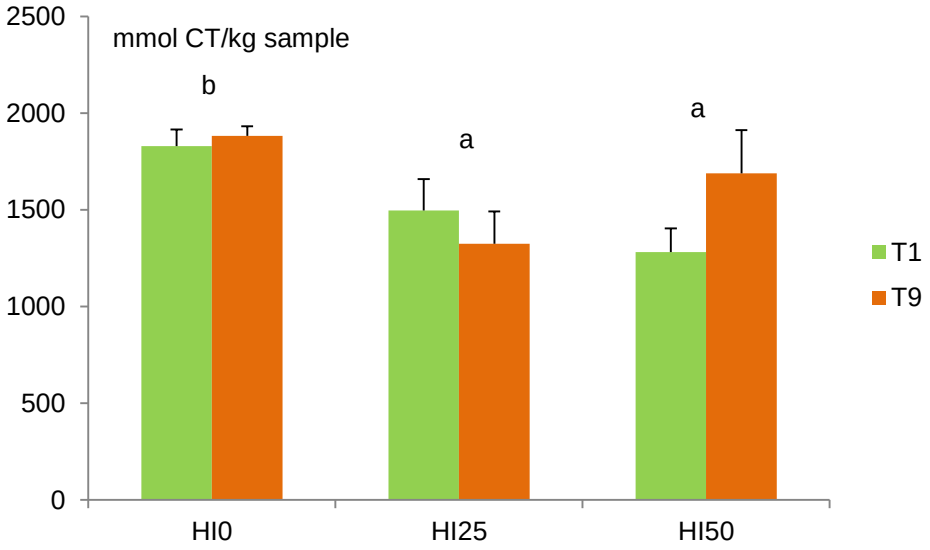

Fig. 14. CT values (mmol CT/kg sample) of fish fed experimental diets, at different times of storage. . Experimental diets with different letters are significantly different ($p < 0.05$).

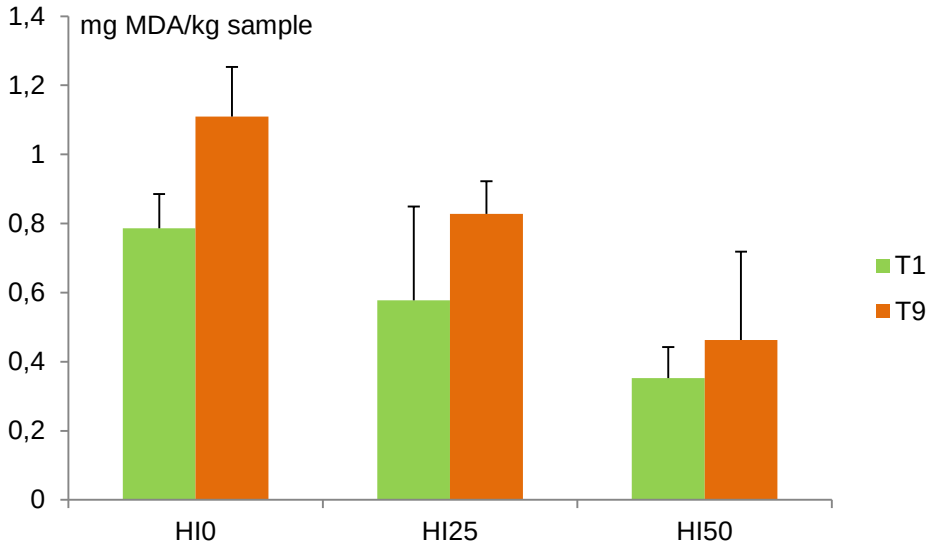

Fig. 15. TBARS values (mg MDA/kg sample) of fish fed experimental diets, at different times of storage. No significant effect of experimental diets was observed.

Total and free amino acids

Data regarding TAAs composition and free FAAs of raw fillets of fish fed the experimental diets are presented in **Table 12** and **13**, respectively. Concerning to TAA, only lysine was significantly ($p < 0.05$) affected by experimental diets. Regarding to FAAs, the EAAs significantly affected by experimental diets were arginine ($p < 0.05$) and methionine ($p < 0.01$), whereas non-EAAs significantly affected by experimental diets were asparagine ($p < 0.01$) and serine ($p < 0.01$).

Table 12. Total amino acids composition (as %) of raw fillets of fish fed the experimental diets.

		HI0	HI25	HI50	Significance	RSD[1]
Essential AA	Arginine	3.886	3.853	3.886	ns	0.103
	Histidine	4.840	4.057	3.047	ns	1.650
	Isoleucine	5.320	5.365	5.415	ns	0.373
	Leucine	8.214	8.480	8.292	ns	0.561
	Lysine	7.240[b]	8.964[ab]	7.926[a]	*	0.814
	Methionine	2.171	2.049	2.008	ns	0.196
	Phenylalanine	3.857	3.695	3.479	ns	0.384
	Threonine	3.332	3.543	3.621	ns	0.251
	Valine	6.246	6.369	6.437	ns	0.335
Non-essential AA	Alanine	8.858	8.884	9.222	ns	0.309
	Asparagine	10.018	10.402	10.992	ns	0.686
	Cystine	0.073	0.075	0.074	ns	0.012
	Glutamine	13.706	14.284	15.022	ns	1.052
	Glycine	10.748	9.979	8.707	ns	1.278
	Hydroxyproline	0.699	0.321	0.266	ns	0.517
	Proline	5.219	5.301	4.905	ns	0.461
	Serine	2.630	2.697	2.758	ns	0.224
	Taurine	0.497	0.534	0.504	ns	0.079
	Tyrosine	2.447	2.375	2.213	ns	0.355

[1]Residual Standard Deviation; ns: not significant; *: $p < 0.05$.

Table 13. Free amino acids composition (as %) of raw fillets of fish fed the experimental diets.

		HI0	HI25	HI50	Significance	RSD[1]
Essential AA	Arginine	1.727[a]	1.099[b]	1.312[b]	*	0.260
	Histidine	13.000	16.320	14.676	ns	4.122
	Isoleucine	0.854	0.555	0.488	ns	0.567
	Leucine	1.533	0.986	0.862	ns	0.734
	Lysine	3.525	2.084	1.467	ns	1.170
	Methionine	0.000[c]	0.201[b]	0.393[a]	**	0.104
	Phenylalanine	0.575	0.523	0.413	ns	0.330
	Threonine	2.643	2.818	2.426	ns	0.794
	Valine	1.981	1.358	1.501	ns	0.721
Non-essential AA	Alanine	1.981	1.501	1.358	ns	0.721
	Asparagine	1.481[b]	2.626[a]	3.060[a]	**	0.578
	Cystine	0.000	0.000	0.000	-	-
	Glutamine	3.775	3.253	3.535	ns	0.695
	Glycine	29.908	31.439	33.795	ns	4.182
	Hydroxyproline	2.785	1.991	2.391	ns	1.122
	Proline	2.461	2.365	2.407	ns	0.601
	Serine	3.500[b]	3.349[b]	4.771[a]	**	0.575
	Taurine	27.229	26.071	24.835	ns	1.950
	Tyrosine	1.041	0.918	0.851	ns	0.228

[1]Residual Standard Deviation; ns: not significant; *: $p < 0.05$; **: $p < 0.01$

Discussion

In order to contribute for the development of more sustainable aquafeeds for intensive aquaculture production, particularly of carnivorous fish species, like *Oncorhynchus mykiss*, it is necessary to find feasible alternatives to FM. Insects have been considered a potential substitute and their inclusion in fish diets has been hardly studied. In this context, several researches have shown that *Hermetia illucens* prepupae could be a good candidate to replace partially or totally the FM in fish diets (Sánchez-Muros et al., 2014; Tran et al., 2015).

The value and the feasibility of an alternative feedstuff to fishmeal cannot simply be evaluated by its ability to maintain growth, as fish market value for human consumption depends, in large part, on the perceived quality (Amberg and Hall 2008; Sealey et al., 2011). However, regarding these aspects, little research has been conducted to date.

The present study demonstrated that fish fed HI25 diet reached the highest final body weight, followed by fish fed HI50 and HI0 diets. Thus, HI prepupae could be used to replace up to 50% of FM in *O. mykiss* diets without compromise the fish growth. Contrarily, Sealey et al. (2011) observed that growth of fish fed 25 or 50% HI based diets significantly reduced as compared to that of the fish fed HI0 control diets. Nonetheless, St-Hilaire et al. (2007a) verified that despite total weight gain of fish fed diets containing 50% of HI prepupae was lower than HI0, there was no significant difference in total weight gain between the fish fed HI0 and those fed 25% HI prepupae diet. Another study, conducted by Lock et al. (2014) to examine the effects of replacing dietary FM with HI prepupae in Atlantic salmon (*Salmo salar*), demonstrated that diets containing 25, 50 and 100% of HI prepupae performed equally well as the HI0 diet and an increase in feed conversion efficiency was observed. This difference of results may be due to variations on HI prepupae meal composition, largely influenced by the diet utilised for feeding larvae and by the consequent quality as well as by the processing technological treatment of insect meal production and the feeding habit of the species (Lock et al., 2014). Together these aspects may result in a wide variability of nutrients of HI meal. Growth depression may also be due to palatability issues. However, none of these authors reported reduction of voluntary feed intake due to dietary FM replacement by HI.

The importance of slaughter traits for the fish industry is well known and morphometric traits can be useful selection criteria of fish for human consumption. In the present study, the dietary inclusion of HI did not affect the morphometric and body traits of experimental fish and to date there is no scientific evidence of the effect of dietary inclusion of HI in fish diets on marketable traits of fish.

Colour is an important quality parameter that can be also considered as an index of animal welfare in aquaculture species (Ocaño-Higuera et al., 2011; Suaréz et al., 2014), constituting one of the main qualities sought by consumers (Bugeon et al., 2010). In the present study, experimental diets did not significantly affect colour values of gills and of raw or cooked fillets. Comparisons with other studies are difficult since there have been very few investigations where physical characteristics, specifically colour, has been studied in fish, namely in *O. mykiss,* fed diets with inclusion of insects, namely HI prepupae. Nevertheless, it has been established, in several fish species, that higher moisture content of fillets resulted in lower lightness (L*) (Hernandéz et al., 2009) and higher muscle-fat contents resulted in higher lightness (L*) and yellowness (b*) values (Marty-Mahé et al., 2004; Mørkøre et al., 2001; Suaréz et al., 2014). The lipid accumulation in muscle occurs in the intramuscular adipose tissue, which is a non-pigmented and opaque white tissue, resulting in higher lightness (L*) and yellowness (b*) values (Einen et al., 1998; Kause et al., 2011; Marty-Mahé et al., 2004; Regost et al., 2001). Higher moisture content contributes to the creation of refractive indices within the food matrix leading to lower lightness (L*) (Hernandéz et al., 2009). Also it has been established that on the same samples, colour can vary between different parts of the fillet (Skjervold et al., 2001). According to this, the results of present study can be justified by the absence of significant differences in fillets crude lipids content and moisture between fillets from HI0, HI25 and HI50 groups.

Fish colour (at level of skin, gills and fillet) can also be influenced by other factors, such as storage period (Hernandéz et al., 2009). In the present study, the gills colour was not affected by dietary treatment but was greatly affected by storage time. The lightness (L*) and perception of colour (Hue) of gills decreased from T1 to T3 but remained stable after this time, with no significant differences among T3, T6 and T9. The opposite trend occurred for redness (a*), yellowness (b*) and saturation (Chroma) values, this last parameter depending from the chromaticity indexes (redness and yellowness).The change in gill colour during the storage is one of the criteria utilised in the freshness evaluation scheme, adopted in the EU (Reg. 2406/1996), since the colour of gills is largely sensitive to the storage time. In the case of whitefish, like rainbow trout, the colour is described as bright, less coloured, brown, or yellowish,

when the fish are classified for freshness in the Extra, A, B or not admitted categories. Regarding to raw fillets, storage time had a significant effect on lightness (L*), which followed the same decreasing trend as in the gills. These results are not in agreement with the results of research led by Hernandéz et al. (2009) on another fish species (meagre) where, with the exception of redness (a*), all the parameters defining fillet colour showed a significant correlation with storage time and lightness (L*) values showed a slight increase as the storage time progressed. The difference of results can be due to the different fish species as well as to the difference of duration of the trials. The Hernandéz et al. (2009) trial lasted 18 days (20 *post-mortem* days) whilst the present results are relative to a post-mortem period lasting 9 days. In addition, Hernandéz et al. (2009) mentioned that significant differences of lightness (L*) values were observed as of day 7. On the other hand, the storage time conditions, such as temperature among others, may also have influenced the results. Several authors have attributed colour loss in fish muscle during storage to the oxidation of proteins with haemo groups, such as haemoglobin and myoglobin (Chaijan et al., 2006).

Concerning to cooked fillets, storage time had a significant effect on lightness (L*), yellowness (b*) and saturation (Chroma) values, since the fillets showed higher values at T3 in comparison with samples analysed at the other storage times. These results are in agreement with the results of Einen and Thomassen (1998), where at the start of their experiment, increased lightness (L*), redness (a*) and yellowness (b*) values were found, whereas this was not shown at the end of the experiment. Nonetheless, in the present study, was not verified an increase of a* value. Increased lightness (L*) and yellowness (b*) values in cooked compared to raw fillets are in agreement with previous studies of Choubert and Baccanaud (2006), Larsen et al. (2011) and Martelli et al. (2014). This increase can be due to heat-induced oxidation of conjugated double bonds of carotenoid molecules, which leads to discolouring of flesh (Martelli et al., 2014). Moreover, according to Larsen et al. (2011), protein aggregation probably increases opacity and the light that enters the surface has less chance of being selectively absorbed. In contrast, Choubert and Baccanaud (2010) found a decrease in lightness (L*) and yellowness (b*) values after dry and moist cooking of *O. mykiss* and associated it with loss of yellow component. Body weight significantly affected redness (a*) and yellowness (b*) values of raw fillets and perception of colour values (Hue) of both raw and cooked fillet, confirming the opportunity of including body weight as covariate in the statistical model utilized to analyse the data, with the aim to better highlight the effect of the different diets utilised.

As an index, pH is important in determining the quality of fish, and can be used as a guide of freshness because of its influence on bacterial growth (Pacheco-Aguilar et al., 2000; Obemeata et al., 2011). According to Obemeata et al. (2011), the higher the pH, the faster the growth of bacteria. The results of present study demonstrated that diet did not have significant effect on pH of fillets, whereas, according to a physiological behaviour of this parameter, the time of storage had, since their values had a tendency to increase over time of refrigerated storage. These results are in agreement with those of Al-Ghanim (2016), Andersen et al. (1997), Dawood et al. (1986), Lesiow et al. (2009) and Mørkøre et al. (2002). The increase in pH of fillets may express the accumulation of alkaline compounds such as ammonia and trimethylamine, mainly derived from microbial actions during fish muscle spoilage (Al-Ghanim, 2016; Susanto et al., 2011). Moreover, the activation of the endogenous proteolytic enzymes (calpains and cathepsins), which contribute to autolysis of fish myofibrils producing polypeptides and oligopeptides, may also contribute for the increase of pH (Ahmed et al., 2015). Indeed, the decomposition of the nitrogenous compounds by endogenous or microbial enzymes increases the volatile compounds responsible of the increase of pH (Al-Ghanim, 2016). Nonetheless, some authors observed an acidification of fillets over the storage time (Lefevre et al., 2015; Lopéz-Luna et al., 2014; Obemeata et al., 2011). This discrepancy between results may be due to the difference in conditions of storage among studies (for example the temperature of storage), as well as the difference in tissue glycogen content, and to the stress suffered by fish during the handling before slaughtering and at slaughtering, and consequently to the amount of lactic acid produced after death by glycogenesis, a process that reduces tissue pH (Concollato et al., 2014). It is worth to note that the intensity of pH changes depends mainly on the storage temperature and that pH values above 7.1 are indicative of decomposition (Hernandéz et al., 2009).

According to Cheng et al. (2014), texture and structure of fish muscle are important freshness and quality attributes. The structure of fish fillet is ensured by myotomes which are linked by intramuscular connective tissue. Collagen is the main constituent of the connective tissue, amounting from less than 3 to 10% of the protein. Collagen plays a vital role in maintaining fillet integrity and muscle cohesiveness (Sikorski et al., 1984; Cheng et al., 2014). Fish flesh contains approximately 3% of collagen, or less, that appears to be even lower in salmonid muscle, resulting in a very tender product (Fauconneau et al., 1992).

In the present study, shear force of the raw fillets was not significantly affected by the diet. Nonetheless, the flesh texture has been associated to the muscle lipid accumulation. Lefevre et al. (2015) verified that low fat of raw flesh of *O. mykiss* presented higher values for mechanical resistance. These authors reported that effect of muscle lipid content on texture could be explained by a higher proportion of intramuscular adipocytes, which form a mechanically less resistant tissue, compared to tissue rich in fibrous proteins, such as myotomes and connective tissue, which are rich in collagen. Indeed, some authors reported an effect of muscle lipid deposition level on fillet texture as those obtained for *O. mykiss* and *S. salar* (Andersen et al., 1997; Johansson et al., 2000; Robb et al., 2002). However, other authors did not observe an effect of muscle lipid content on textural parameters in *O. mykiss* (Bjerkeng et al., 1997; Rasmussen et al., 2000; Regost et al., 2001; Rørå et al., 1998). In the present study, even though the mechanical resistance was lower for fillets of fish fed the HI0 control diet (but not significantly different) than that of the fillets from fish fed the other diets, no differences were observed on muscle lipid content among the treatments. And this supports the lack of difference in textural properties of fillets from fish fed the three tested diets. Different factors may contribute to the variability in results found in the available literature, including physical factors (fish species, age and size of fish, sample heterogeneity, and gaping), chemical factors (muscle moisture, lipid and collagen content and distribution) and differences in the *post mortem* treatments and processing (storage time and temperature, freezing, chilling, high-pressure processing (HPP), salting and smoke; Cheng et al., 2014).

On the other hand, the effect of storage time on texture of raw fillets was significant, where shear force presented a decreasing trend during refrigerated storage. Despite the differences in the experimental conditions and the instrumental parameters among different studies, these results can be considered in agreement with those obtained by several authors (Andersen et al., 1997; Cheng et al., 2014; Delbarre-Ladrat et al., 2006; Lesiow et al., 2009; Martinez et al., 2007; Taylor et al., 2002). Nonetheless, in a recent investigation, the firmness of rainbow trout fillet was evaluated using the variable-blade attachment and the Allo-Kramer shear method; it was observed that the fillet refrigerated at 4 ℃ was firmer at the start of the storage than after preservation for 14 days (Aussanasuwannakul et al., 2012). These results are related to the numerous biochemical modifications that occurred *post-mortem* in the muscle and that contribute to meat tenderization process. During early *post-mortem* period, the proteolytic cleavage of important structural proteins in myofibrils and extracellular matrix, as well as of costamere (Z-disk associated structures that connect

the myofibrils to the sarcolemma) and intermediate filament proteins (proteins involved in inter-myofibrillar linkages), contributes to tissue softening (Taylor et al., 2002; Delbarre-Ladrat et al., 2006). In the later stages of storage period, the structural changes and degradation of connective tissue occur which seem to mainly contribute to the tenderization process of fish flesh (Hernandéz-Herrero et al., 2003; Nishimura et al., 1998). Finally, the degradation of sarcoplasmic proteins releases glycolytic enzymes (Hernandéz-Herrero et al., 2003). Reduced shear force values in cooked compared to raw fillets are in agreement with previous studies of Aussanasuwannakul et al. (2010), Hyldig and Nielsen (2001), Martelli et al. (2014), Mørkøre et al. (2002) and Schubring (2008). This variation in texture of cooked fillets is due to myofibril disintegration (Martelli et al., 2014). The thermal changes in myofibrillar proteins increase toughness, as well as the transformation of collagen into gelatin (Schubring, 2008), which make the flesh more tender since the layered myotomes tend to slide away in response to compression (Martelli et al., 2014).

Water-Holding-Capacity (WHC) can be defined as the amount of water left in the muscle after centrifugation (Andersen and Jørgensen, 2004). When referred to WHC, the amount of expelled fat is also taken into account (Skipnes et al., 2007). WHC can be used as a measure of quality of fish fillets (Rustad, 1992; Olsson et al., 2003). It is an important quality parameter, as it affects both profitability parameters, such as weight change during transportation and storage, drip loss during thawing, weight loss and shrinkage during cooking, and quality parameters such as texture, tenderness and juiciness, which are very important quality factors for the consumer (Irie et al., 1996; den Hertog-Meischke et al., 1997; Duun et al., 2007; Shaviklo et al., 2010). Higher water content in muscle reduces its mechanical strength and makes the fillets over-soft which is not appreciated by consumers, becoming into a problem for the fish industry (Hultmann and Rustad, 2002; Martelli et al., 2014).

In the present study, WHC values were not affected by the diet, but an effect of storage time was observed. The mean value of WHC demonstrated a tendency to increase over the time. These results are in conformity with the results of experiments led by Bao et al. (2007), Duun et al. (2008), Kaale et al. (2014), Olsson et al. (2003) and Suarez et al. (2014). This increase in WHC could be caused by a higher incidence of loosely bound water that was released as drip over time (Bao et al., 2007) or by proteolytic activity in the muscle during storage (Kaale et al., 2014), where limited desmin protein degradation may result in an increased shrinkage of the muscle cells which contributes to the formation of gaps between muscle cells and muscle bundles

and reduced myofibrillar strain resulting in an inflow of extra-myofibrillar water to the myofibrillar space, thus increasing WHC (Offer and Cousins, 1992; Purslow et al., 2001). WHC is also highly influenced by the pH of the muscle. A pH closer to the iso-electric point of the myofibrillar protein will increase the protein-protein attraction, due to less negative charges of the amino acids, and thus reduce the water-holding capacity (Hermansson, 1986; Honikel, 1989).

In the results of present study, the cooking loss (CL) values were significantly affected by the diets utilised. Fish fed by H50 diet had the highest values of CL, followed by HI25 fish (that were not significantly different from the two groups) and finally by HI0. The increased of CL resulted from the reduction of WHC in fish fed the HI50 diet. Nonetheless, comparisons with other studies are difficult since there have been very few investigations on the effect of diet on CL of fish, these results are in agreement with those of Cao et al. (2016), that found that the CL slowly decreased with the extension of storage time. Still, differences may be attributed to higher activity of cathepsin B and L. Indeed, previously it was reported that cooking loss was positively related to the cathepsin B and L activities that play a key role in fish muscle degradation (Gan et al., 2014).

At the end of this study, the fillets proximate composition was unaffected by the dietary HI prepupae inclusion. Though, it has to be remembered that experimental diets were formulated to be isoproteic, isolipidic and isoenergetic. Nonetheless, raw fillets proximate composition was significantly affected by storage time regarding to all chemical parameters, excluding CP. Over the storage time, the increase in water and ash content of raw fillets can be explained as consequence of decrease of lipid content. Instead, proximate composition of the cooked fillets was affected by the time of storage only with regard to total lipids. In both raw and cooked fillets was observed a decrease in content of total lipids. This decrease could be explained by hydrolytic and oxidative reactions. Several studies on horse mackerel, sardine and Nile tilapia have shown that, during refrigerated storage, triacylglycerol content decreases, along with the increase of free fatty acids, peroxide and thiobarbituric acid reactive substances, indicating that lipid hydrolysis and oxidation took place (Chaijan et al., 2006; Losada et al., 2005; Sae-leaw et al., 2013).

In the present study, the composition of FA was the feature that was most affected by inclusion of HI prepupae in diets. Almost all FAs were significantly affected

by diet and it can be perceived that the fatty acid profile of fillets from each treatment group follows the profile of the diet consumed. In agreement with Bell et al. (2001), Stubhaug et al. (2007), Sealey et al. (2011), Thomassen and Røsjø (1989) and Torstensen et al. (2004), high correlations have been observed between the general FA composition of the diets and the FA composition of the fish muscle in almost all species studied. The HI prepupae meal used in this trial trial was very reach in SFA (more than 71.8% of total fatty acids) and as a consequence the content of SFA of the diet increased together with the increase of inclusion of insect meal, indeed HI0, HI25 and HI50 diet contained, respectively, 24.5, 36.5 and 47.1% of SFA. The final content of these FAs in the fish fillets for the HI0, HI25 and HI50 were, respectively, 23.1, 29.6 and 36.6%. These results are in agreement with previous studies, where fish fed diets with a low content of SFA had lower concentration of this class in the muscle (Bell et al., 2001; Greene et al.,1990; Turchini et al., 2003). The lauric acid (C12:0) has particular interest among the SFA. It is a medium-chain fatty acid (MCFA), constituent of medium-chain triacylglycerol (MCT). MCFAs follow a different metabolic pathway compared to long-chain fatty acids. They can directly enter the portal vein, accelerating their uptake and oxidation by the liver, leading to a limited storage of this class in the different tissues (Lhuillery et al., 1988). In the present study, lauric acid accounted for 46.8% of total FAs of insect meal, and the content of lauric acid in the diet was 3.3% in HI0, 14.4% in HI25 and 24.4% in HI50. Looking at the final content of this FA in the fillets, it was negligible in HI0 individuals, while it was substantial for HI25 (6.1%) and HI50 (11.8%). The storage of this FA in the tissues was therefore substantial, in agreement with Figueiredo- Silva et al. (2012). Besides SFA, the group of PUFAn3 was also strongly affected by the diet, in particular in regard to eicosapentaenoic acid (C20:5n3; EPA) and docosapentaenoic acid (C22:5n3; DHA). In insect meal, EPA was only 0.6% and DHA only 0.3% of TFA. Nevertheless, in the formulated diets, EPA accounted for 10.4% in HI0, 7.7% in HI25 and 5.9% in HI50. The content of DHA in the diets were even lower (1.5, 1.2 and 1.0%, in HI0, HI25 and HI50 diet, respectively). Therefore, even if the lipid source of the three diets was the same (FO), the residual fat deriving from the protein source (exclusively FM or FM and HI in different percentages) led to relevant differences in the quality of lipids of the feeds. The FA composition of the fillets reflected the differences among the diets, as EPA accounted for 4.4%, 3.3% and 2.3% in individuals fed HI0, HI25 and HI50 diets, respectively. Similarly, DHA was contained in the amounts of 19.5%, 16.6% and 13.3% in raw fillet of fish fed the three diets, respectively. The amounts of EPA and DHA in fish fed these diets are in agreement with those reported in literature (Steffens, 1997). After the cooking process,

the FA composition of fillets was very similar to the FA profile of raw fillets. The only slight change occurred was an increase in PUFAn3 and a decrease in MUFA, while SFA and PUFAn6 remained unchanged, which are in agreement with Ashgari et al. (2013).

For both raw and cooked fillet, the results of the present study revealed that the time of storage had a significant effect only on C18:0 and C16:1n7 but no specific trend of decrease or increase was evident. The effect of the interaction between diet and time of storage was not significant, except for C18:0. It has to be noticed also that body weight significantly affected the incidence of most of the fatty acids, confirming the opportunity of including body weight as covariate in the statistical model utilized to analyse the data to better clarify the possible influence of the diet.

The AI and TI were proposed by Ulbricht and Southgate (1991) to characterize the atherogenic and thrombogenic potential of FA. The lower values of both indices demonstrate the better nutritional/functional quality of FA, as diets with low AI and TI values could reduce the potential risk of coronary heart diseases (Hosseini et al., 2014). In the present study, both AI and TI were significantly affected by the diet, since both these indexes increased with the level of inclusion of HI prepupae in the diets. In raw fillets these values ranged from 0.37 to 0.73 for AI and from 0.13 to 0.23 for TI. In cooked fillets these values ranged from 0.37 to 0.77 for AI and from 0.13 to 0.20 for TI. Suárez et al. (2014), in an experimental trial on *O. mykiss* reared in different conditions, reported values ranging from 0.49 to 0.62 for AI and from 0.23 to 0.28 for TI. In other words, TI values of the present study were lower than those found by Suarez et al. (2014) for all treatments, whereas AI values for control diet (0.37) was lower and for HI50 (0.77) the values were higher than those reported by Suárez et al. (2014). Based on AI and TI values reported for different seafoods (AI ranging from 0.33 to 2.37 and TI ranging from 0.01 to 1.18), the results of the present study showed that the values found can be considered acceptable, even when a high percentage of FM was replaced by HI meal (Filho et al., 2010; Kalogeropoulos et al., 2004; Rosa et al., 2007; Turan et al., 2007).

The cooking process did not lead to changes neither in AI nor in TI. Nonetheless, in a study on Kutum roach the boiling had no significant effect on TI, but it led to a significant increase in AI (Hosseini et al., 2014). Concerning to storage time, this parameter only affected significantly TI of raw fillets, as it slightly decreased from T1 to T3, but it remained stable from T3 to T9, as consequence of the specific evolution showed by the fatty acids included in the formula, during the refrigerated storage.

Regarding to HH ratio, an index proposed by Santos-Silva et al. (2002) to characterize the quality of lipid fraction in limiting the cholesterol level of consumers, the results of the present research showed that the diet had a significant effect, since the cooked fillets presented values significantly decreasing from 3.14 (HI0) to 2.76 (HI25) and 2.38 (HI50), with the increase of HI inclusion in the diet. It should be remembered that higher values of HH ratio would be desirable, and then the diet HI0 had the most favorable effect on this parameter. The values of HH ratio of cooked and raw fillets were similar to each other. These results are not in agreement with those obtained by Asghari et al. (2013), that for this parameter registered values significantly decreased after cooking. Nonetheless, in previous studies the HH ratio values for different species were found ranging from 0.25 to 3.59 (Filho et al., 2010; Hosseini et al., 2014; Testi et al., 2006). Concerning to PUFAn3/PUFAn6 and PUFAn6/PUFAn3, these ratios are considered good indicators of the relative nutritional values of foods. There is an increasing evidence to suggest that n3 HUFA, such as EPA and DHA, are extremely beneficial to human health, together with a balanced dietary PUFAn3/PUFAn6 or PUFAn6/PUFAn3 ratios (Ruxton et al., 2005; Sinclair et al., 2007). As fish is believed to be the major contributor of EPA and DHA in the human diet and its PUFAn3/PUFAn6 ratio is remarkable, its health beneficial properties need to be maintained. In the present study the values of PUFAn3/PUFAn6 ratio decreased with the increase of inclusion of HI in the experimental diets and, obviously, the opposite occurred for PUFAn6/PUFAn3 ratio. According to current WHO recommendations, the overall daily ratio of PUFAn3/PUFAn6 in the human diet should be no higher than 1:5 (at least 0.2) and the ratio of PUFAn6/PUFAn3 should be less than 15. According to this, the values found in this study could be considered satisfying and all values were found still in the range of values found for freshwater fish. Moreover, the PUFAn3/PUFAn6 ratio slightly increased after cooking reaching 3.20 for HI0, 2.72 for HI25 and 2.15 for HI50 samples. The value of this ratio calculated by Asghari et al. (2013) was only 1.23 for the raw *O. mykiss* fillets and did not change for the cooked fillets. The PUFA/SFA ratio aims to demonstrate if the analysed food is a good source of PUFA. The minimum value recommended for humans is 0.45 (Domingo, 2007). In the present research, the calculated ratio was 1.82 in raw fillets and 1.87 in cooked fillets fed the control diet. In a previous study, Asghari et al. (2013) verified lower mean value (0.91) in raw fillets, which slightly decreased to 0.87 in the cooked fillets. The effect of the insect meal inclusion in the diet was significant in both raw and cooked fillet, but even though the ratio PUFA/SFA decreased with the increase of insect meal inclusion (to 1.29 and 0.88

in cooked fillets from HI25 and HI50 groups, respectively), the PUFA/SFA ratio was still above the recommended value.

Lipid oxidation is an important factor that determines the shelf life of fish and is the main cause of reducing flesh quality (Gan et al., 2014; Secci et al., 2015). Reaction products from lipid oxidation have a negative effect on the sensory quality of fillet and this can lead to complains from the consumers and consequently reduce the sales. Moreover, the chemical products generated by the lipid oxidation may also have negative effects on consumer health (Rustad, 2010; Secci et al., 2015). Different methods to determine the degree of lipid oxidation can be divided in two main groups, methods that determine the primary oxidation products and methods that measure the secondary oxidation products. The most common parameters useful to analyse the primary oxidation products are the peroxide value (PV) and the conjugated dienes (Rustad, 2010). Due to rapid polymerization of EPA and DHA compared with the formation of stable peroxides of these fatty acids, PV is reported to be an unreliable indicator of lipid peroxidation in fish (Falch, 2005). Conjugated diene hydroperoxides are formed when PUFA oxidize. The fatty acid chain then contains a structure with alternating simple and double bonds. Methods that measure the secondary oxidation products are most useful as a measure of lipid oxidation for lipids with a low level of oxidation. These methods determine the presence of aldehydes, which are secondary oxidation products and the determination of TBARS is a common analytical method to determine these products (Rustad, 2010). There are many published methods to determine TBARS but all of them are based on the pink colour absorbance formed by reaction between TBA and oxidation products of polyunsaturated fatty acids. The results of the present study revealed that lipid oxidation of fillets demonstrated a tendency to decrease with the higher content of HI prepupae in the diets. Even total lipid content of fillets has been superior (but not significantly different) along the inclusion of HI prepupae in experimental diets. Present results may be due to the difference of FA profile of fillets from fish fed different diets, since there is a geometrical strict relationship between susceptibility of FA to peroxidation and its degree of unsaturation (Gray, 1977). Indeed, different authors have observed that fish oil in the diet increases the susceptibility to FA peroxidation and the production of TBARS in rainbow trout (Alvarez et al., 1998; Tuchini et al., 2013) as well as in other fish species (Stephan et al., 1995, Du et al., 2008; Ahmad et al., 2013). Moreover, the increase of lipid oxidation products along storage time observed in the present research is in agreement with recent studies of Barnett et al. (2014), Cardenia et al. (2013), Secci et

al. (2015) and Simitzis et al. (2014). Additionally, data in the present study showed that TBARS values after 9 days of storage were much lower than the threshold of 8 mg MDA kg^{-1}, which is the limit of acceptability proposed for most fish species (Secci et al., 2015).

Fish proteins are considered of high-quality because of their balanced content in amino acids especially in all the EAA, necessary for physical and mental well-being. The total EAA profile gives information on the nutritional value of fish flesh (Aristoy and Toldrá, 2010; Gan et al., 2014). It is well known that fish require a well-balanced amino acid profile in feeds to achieve an optimal growth (Berge et al., 1999; Peres and Oliva-Teles, 2009; Sánchez-Muros et al., 2014). Furthermore, emerging evidence from studies with both aquatic and terrestrial animals shows that many AA regulate key metabolic pathways that are crucial to maintenance, reproduction and immune responses (Li et al., 2008). Some AA may also be found in free form (FAA), which contribute to fish taste and indirectly to aroma by generation of volatile compounds and to texture (Aristoy and Toldrá, 2010; Li et al., 2008). FAAs initiate important changes at early *post-mortem* and during storage and can be very useful as quality indices of processing and storage. Thus, the analysis of EAA in fish is important for the evaluation of both the nutritive value and the sensory quality of fish (Aristoy and Toldrá, 2010; Gan et al., 2014). Concerning the protein-bounded AA, in the present study only lysine was significantly affected by the experimental diets, since its content was higher in HI25 followed by HI50 and HI0. Nonetheless, comparisons with other studies are not possible because no literature where AA profile has been studied in fish, namely *O. mykiss*, fed diets with inclusion of insects, namely HI prepupae, was found. Thus, to assess this increase of lysine content, further studies should be performed. Regarding to FAA, arginine and methionine were the EAA significantly affected by experimental diets, where arginine content was higher in HI50 followed by HI0 and HI25 and methionine content increased with the increase of inclusion of HI prepupae in the experimental diets. In spite of comparisons with other studies are difficult, Wang et al. (2015) suggested that arginine increased muscle firmness through increment glutamine and collagen content in fish. Moreover, the results of study led by Wang et al. (2015) also suggested that arginine could enhance the WHC of fish flesh, improving their quality. Nonetheless, no more literature was found and further studies should be performed. Concerning to non-EAA in free form, asparagine and serine were significantly affected by experimental diets, where asparagine increased with the increase of inclusion of HI prepupae meal in experimental diets and serine content was higher in HI50 followed by HI0 and HI25. As above mentioned, comparisons with other

studies are difficult and further studies should be performed. Still, in the trial led by Jiang et al. (2016) was assessed that serine confers desirable taste, namely sweetness, improving quality attributes, namely flavour, of fish flesh. In conformity with this, the high amount of serine in HI50 observed in the present study may improve the flesh quality of *O. mykiss*.

Conclusion

The future of aquaculture nutrition will depend on the search for alternative protein sources for FM replacement as current inclusion rates threaten the expansion of the industry. To our knowledge, this is the first study to evaluate the interaction of dietary inclusion of *H. illucens* meal and physical characteristic and quality traits of fish flesh. The results of present study indicate that, irrespectively the dietary replacement level of fishmeal by *H. illucens* meal, no detrimental effects on morphometric, marketable and physical characteristics, including colour, pH, texture and water-holding capacity of fish flesh were reported.

Nonetheless, increasing inclusion of *H. illucens* meal led to a increase of cooking loss values, while proximate composition of raw and cooked fillet were not affected by the diet. Contrarily, flesh fatty acid profile, and consequently the quality indexes TI, AI, HH, PUFAn3/PUFAn6 and PUFA/SFA, were strongly affected by the substitution of fishmeal with insect meal. Saturated fatty acids increased with the inclusion of *H. illucens*, while MUFA and LC-PUFA (particularly EPA and DHA) decreased with the inclusion of insect meal in the diet. Interestingly, the muscle lipid oxidation products decreased with the inclusion of *H. illucens* in the diet. As expected, the increase of the storage time had a negative effect on almost all tested variables. In conclusion, the results of this study were very promising: *Hermetia illucens* prepupae meal seems to be a good innovative protein source for farmed *O. mykiss*. Further studies are required to enhance the nutritional profile of *H. illucens* meal, especially that related to the fatty acid profile. Overall, it can be concluded that the dietary fishmeal replacement by *H. illucens* up to 50% had little effect on the quality traits of rainbow trout. However, an increase of saturated and a decrease of MUFA and LC-PUFA were observed in muscle of fish fed the insect based diets, which results in a decrease of lipid peroxidation.

References

Achionye-Nzeh, C.G., Ngwudo, O.S., 2003. Growth response of *Clarias anguillaris* fingerlings fed larvae of *Musca domestica* and soyabean diet in the laboratory. Biosci. Res. Commun. 15, 221–223.

Adewolu, M.A., Ikenweiwe, N.B., Mulero, S.M., 2010. Evaluation of ananimal protein mixture as a replacement for fishmeal in practical diets for fingerlings of *Clarias gariepinus* (Burchell, 1822). Israel. J. Aquacult. –Bamidgeh 62, 237–244.

Ahmad, W., Stone, D.A.J., Schuller, K.A., 2013. Dietary fish oil replacement with palm or poultry oil increases fillet oxidative stability and decreases liver glutathione peroxidase activity in barramundi (*Lates calcarifer*). Fish Physiol. Biochem. 39, 1631-1640.

Ahmed, Z., Donkor, O., Street, W.A, Vasiljevic, T., 2015. Calpains- and cathepsins-induced myofibrillar changes in post-mortem fish: Impact on structural softening and release of bioactive peptides. Food Sci. Technol. 45, 130-146.

Ajani, E.K., Nwanna, L.C., Musa, B.O., 2004. Replacement of fishmeal with maggot meal in the diets of Nile tilapia, *Oreochromis niloticus*. World Aquac. 35, 52–54.

Akiyama, T., Murai, T., Hirasawa, Y., Nose, T., 1984. Supplementation of various meals to fish meal diet for chum salmon fry. Aquaculture 37, 217–222.

Alegbeleye, W.O., Obasa, S.O., Olude, O.O., Otubu, K., Jimoh, W., 2012. Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L.) for African catfish *Clarias gariepinus* (Burchell. 1822) fingerlings. Aquac. Res. 43, 412–420.

Al-Ghanim, K.A., 2016. Effect of different storage temperatures on chemical composition and sensory attributes of the flesh of *Cyprinus carpio* and *Clarias gariepinus*. Pakistan J. Zool. 48(2), 305-310.

Alvarez, M.J., Lopez-Bote, C.J., Diez, A., Corraze, G., Arzel, J., Dias, J., Kaushik, S.J., Bautista, J.M., 1998. Dietary fish oil and digestible protein modify susceptibility to lipid peroxidation in the muscle of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*). Brit. J. Nutr. 80, 281-289.

Amberg, S., Hall, T.E., 2008. Communicating risks and benefits of aquaculture: A content analysis of U.S. newsprint representations of farmed salmon. J. World Aqua. Soc. 39, 143-157.

Andersen, C.M., Jørgensen, B.M., 2004. On the relation between water pools and water holding capacity in cod muscle. J. Aquat. Food Prod. Technol. 13 (1), 13-23.

Andersen, U.B., Thomassen, M.S., Rørå, A.M.B., 1997. Texture properties of farmed rainbow trout (*Oncorhynchus mykiss*): effects of diet, muscle fat content and time of storage on ice. J. Sci. Food Agricult. 74, 347–353.

Aniebo, A.O., Erondu, E.S., Owen, O.J., 2009. Replacement of fish meal with maggot meal in African catfish (*Clarias gariepinus*) diets. Rev. Cient. UDO Agric. 9, 666–671.

Aniebo, A.O., Odukwe, C.A., Ebenebe, C.I., Ajuogu, P.K., Owen, O.J., Onu, P.N., 2011. Effect of housefly larvae (*Musca domestica*) Meal on the carcass and sensory qualities of the mud catfish (*Clarias gariepinus*). Adv. Food Ener. Sec. 1, 24–28.

AOAC, 2000. Official Methods of Analysis of AOAC International. 17th Association of Official Analytical Chemists, Gaithersburg. Maryland, USA.

Aristoy, M.C., Toldrá, F., 2010. Handbook of seafood and seafood analysis. Essential Amino Acids. CRC Press, Florida. 287-300 pp.

Asghari, L., Zeynali, F, Sahari, M.A., 2013. Effects of boiling, deep-frying, and microwave treatment on the proximate composition of rainbow trout fillets: changes in fatty acids, total protein, and minerals. J. Appl. Ichthyol. 29, 847–853.

Aussanasuwannakul, A., Kenney, P.B., Brannan, R.G., Slider, S.D., Salem, M., Yao, J., 2010. Relating instrumental texture, determined by variable-blade and Allo-Kramer shear attachments, to sensory analysis of rainbow trout (*Oncorhynchus mykiss*) fillets. J. Food Sci. 75(7), 365–374.

Aussanasuwannakul, A., Slider, S.D., Salem, M., Yao, J., Kenney, P., 2012. Comparison of variable-blade to Allo-Kramer shear method in assessing rainbow trout (*Oncorhynchus mykiss*) fillet firmness. J. Food Sci., 77(9), 335–341.

Bao, H.N.D., Arason, S., Thórarinsdóttir, K.A., 2007. Effects of dry ice and superchilling of quality and shelf life of Arctic charr (*Salvelinus alpinus*) fillets. International J. Food Eng. 3, 1-27.

Barnett, H., Nelson, R., Poysky, F.A. Comparative study using multiple indices to measure changes in quality of pink and Coho salmon during fresh and frozen storage. Available in: http://nwfsc.noaa.gov/assets/25/6225062120041253 47tm208.pdf. (July 2016).

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.

Begun, N.N., Chakraborthy, S.C., Zaher, M., Abdul, M.M., Gupta, M.V., 1994. Replacement of fish meal by low cost animal protein as a quality fish feed ingredients for the Indian major carp, *Labeo rohita*, fingerlings. J. Sci. Food Agric. 64, 191–197.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of fish oil with rape seed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr. 131, 1535–1543.

Berge, G.E., Bakke-McKellep, A.M., Lied, E., 1999. In vitro uptake and interaction between arginine and lysine in the intestine of Atlantic salmon (*Salmo salar*). Aquaculture 179, 181-193.

Bjerkeng, B., Refstie, S., Fjalestad, K.T., Storebakken, T., Rùdbotten, M., Roem, A.J., 1997. Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. Aquaculture 157, 297-309.

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

Bondari, K., Sheppard, D.C., 1987. Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). Aquacult. Fish. Manage. 18, 209–220.

Boscolo, W.R., Hayashi, C., Meurer, F., 2001. Fish, meatandbone, poultry by-products and silkworm meals as attractive in diets for Nile tilapia (*Oreochromis niloticus*) fingerlings. Rev. Bras. Zootec. 30, 1397–1402.

Bugeon, J., Lefevre, F., Cardinal, M., Uyanik, A., Davenel, A., Haffray, P., 2010. Flesh quality in large rainbow trout with high or low fillet yield. J. Muscle Foods 21(4), 702–721.

Calvert, C.C., Martin, R.D.A., 1969. Housefly pupae as food for poultry. J. Econ. Entomol. 62, 938-939.

Cao, L., Niu, L., Sun, X., Fan, Y., Huang, Y., Lai, K., 2016. Changes in freshness and thermal processing properties of grass carp (*Ctenopharyngodon idellus*) fillet during ice storage. Food Sci. 37(12), 267-272.

Cardenia, V., Rodriguez-Estrada, M.T., Baldacci, E., Lercker, G., 2013. Health-related lipids components of sardine muscle as affected by photooxidation. Food Chem. Toxicol. 57, 32–38.

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

Cheng, J., Sun, D., Han, Z., Zeng, X., 2014. Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: a review. Compr. Rev. Food Sci. Food Saft. 13, 52-61.

Choubert, G., Baccanaud, M., 2006. Colour changes of fillets of rainbow trout (*Oncorhynchus mykiss* W.) fed astaxanthin or canthaxanthin during storage under controlled or modified atmosphere. Food Sci. Technol. 39, 1203-1213.

Choubert, G., Baccaunaud, M., 2010. Effect of moist or dry heat cooking procedures on carotenoid retention and colour of fillets of rainbow trout (*Oncorhynchus mykiss*) fed astaxanthin or canthaxanthin. Food Chem. 119, 265-269.

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

Concollato, A., Parisi, G., Olsen, R.E., Kvamme, B.O., Slinde, E., Dalle Zotte, A., 2014. Effect of carbon monoxide for Atlantic salmon (*Salmo salar* L.) slaughtering on stress response and fillet shelf life. Aquaculture 433, 13–18.

Dawood, A.A., Roy, R.N., Williams, C.S., 1986. Quality of rainbow trout chilled- stored after postcatch holding. J. Sci. Food Agricult. 37, 421–427.

Delbarre-Ladrat, C., Cheret, R., Taylor, R., Verrez-Bagnis, V., 2006. Trends in postmortem aging in fish: understanding of proteolysis and disorganization of the myofibrillar structure. Crit. Rev. Food Sci. Nutr. 46, 409-421.

den Hertog-Meischke, M.J.A., van Laack, R.J.L.M., Smulders, F.J.M, 1997. The water-holding capacity of fresh meat. Vet. Quart. 19 (4), 175-181.

Dheke, S., Gubhaju, R.S., 2013. Growth response of rainbow trout (*Oncorhynchus mykiss*) on substitution of shrimp meal by different protein sources. Nepalese J. Zool. 1, 24–29.

Diclaro, J.W., Kaufman, 2015. Black soldier fly *Hermetia illucens* Linnaeus (Insecta: Diptera: Stratiomyidae). EENY 461.

Diener, S., Zurbrügg, C., Gutiérrez, F.R., Nguyen, D.H., Morel, A., Koottatep, T., Tockner, K., 2011. Black Soldier Fly Larvae for Organic Waste Treatment – Prospects and Constraints. Proc. WasteSafe 2011 – 2nd Int. Conf. Solid Waste Manag. Dev. Ctries. 52, 978–984.

Domingo, J.L., 2007. Omega-3 fatty acids and the benefits of fish consumption: is all that glitters gold?. Environ. Int. 33, 993–998.

Dong, G.F., Yang, Y.O., Song, X.M., Yu, L., Zhao, T.T., Huang, G.L., Hu, Z.J., Zhang, J.L., 2013. Comparative effects of dietary supplementation with maggot meal and soybean meal in gibel carp (*Carassius auratus gibelio*) and dark barbell catfish (*Pelteobagrus vachelli*): Growth performance and antioxidant responses. Aquac. Nutr. 19, 543–554.

Du, Z.Y., Clouet, P., Huang, L.M., Degrace, P., Zheng, W.H., He, J.G., Tian, L.X., Liu, Y.J., 2008. Utilization of different dietary lipid sources at high level in herbivorous grass carp (*Ctenopharyngodon idella*): mechanism related to hepatic fatty acid oxidation. Aquacult. Nutr. 14, 77-92.

Duun, A.S., Rustad, T., 2007. Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. Food Chem. 105 (3), 1067-1075.

Ebenso, I.E., Udo, M.T., 2003. Effect of live maggot on growth of the Nile perch, *Oreochromis niloticus* (Cichlidae) in South Eastern Nigeria. Glob. J. Agric. Sci. 2, 72–73.

Eide, O., Borresen, T., Strom, T., 1982. Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from small fatty fish species. J. Food Sci. 47, 347-349.

Einen, O., Thomassen, M.S., 1998. Starvation prior to slaughter in Atlantic salmon (*Salmo salar*). II. White muscle composition and evaluation of freshness, texture and colour characteristics in raw and cooked fillets. Aquaculture 169, 37–53.

Emehinaiye, P.A., 2012. Growth Performance of *Oreochromis niloticus* Fingerlings Fed with Varying Levels of Migratory Locust (*Locusta migratoria*) Meal. Aquacult. Fish. Manage., Federal University of Agriculture, Abeokuta, Nigeria.

EUMOFA. (2015). The EU Fish Market. European Market Observatory for Fisheries and Aquaculture Products, Brussels.

Fakayode, O.S., Ugwumba, A.A.A., 2013. Effects of replacement of fish meal with palm grub (*Oryctes rhinoceros* (Linnaeus, 1758)) meal on the growth of *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* (Valenciennes, 1840) fingerlings. J. Fish. Aquat. Sci. 8, 101–107.

Falch, E., 2005. Lipids from residual fish raw material, in Department of Biotechnology. Norwegian University of Science and Technology. 206.

FAO. 1988. Definition of aquaculture, Seventh Session of the IPFC Working Party of Expects on Aquaculture, IPFC/WPA/WPZ, p.1-3, RAPA/FAO, Bangkok.

FAO. 2014. *The State of World Fisheries and Aquaculture 2016. Opportunities and Challenges*. Rome. 223 pp.

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

FAO. 2016b. http://www.fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en (February, 2016).

FAO, 2016c. http://www.fao.org/fishery/affris/species-profiles/rainbow-trout/nutritional-requirements/en/. (February, 2016)

Fasakin, E.A., Balogun, A.M., Ajayi, O.O., 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquac. Res. 34, 733–738.

Fauconneau, B., Chmaitilly, J., Andreå, S., Cardinal, M., Cornet, J., Vallet, J.L., Dumont, J.P., Laroche, M., 1992. Characteristics of rainbow trout flesh. I.

Chemical composition and cellularity of muscle and adipose tissues. Sci. Aliment. 13, 173-187.

Figueiredo-Silva, A.C., Kaushik, S., Terrier, F., Schrama, J.W., Médale, F., Geurden, I., 2012. Link between lipid metabolism and voluntary food intake in rainbow trout fed coconut oil rich in medium-chain TAG. J. Nutr. 107, 1714–1725.

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. J. Am. Oil Chem. Soc. 87, 1461–1467.

FishBase, 2016. http://www.fishbase.org/summary/239 (February, 2016).

Folch, J., Lees, M., Sloane-Stanley, G. H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.

Gan, L., Jiang, WD., Wu, P., Liu, Y., Jiang, J., Li, SH., Tang, L., Kuang, SY., Feng, L., Zhou, XQ., 2014. Flesh quality loss in response to dietary isoleucine deficiency and excess in fish: a link to impaired Nrf2-dependent antioxidant defense in muscle. Available in: http://dx.doi.org/10.1371/journal.pone.0115129. (July 2016).

Gao, S., Yin, N., Zhou, F., Li, H., Zhou, J., Wang, R. J., Shao, Q.J., 2013. Evaluation of pea proteins and poultry protein as fish meal alternatives in the diets for juvenile black sea bream, *Acanthopagrus schlegelii*. Aquac. Nutr. 19(3), 278-288.

Gasco L., Schiavone A., Parisi G., 2015. Insects in fish and poultry feeding. Tecnica Molitoria, 66 (12).

Gasco, L., Belforti, M., Rotolo, L., Lussiana, C., Parisi, G., Terova, G., Roncarati, A., Gai, F., 2014a. Mealworm (*Tenebrio molitor*) as a potential ingrediente in practical diets for rainbow trout (*Oncorhynchus mykiss*). In: Vantomme, P., Munke, C.,van Huis, A.(Eds.), 1st International Conference “Insects to Feed the World”. Wageningen University, Ede-Wageningen, The Netherlands, p.69.

Gasco, L., Gai, F., Piccolo, G., Rotolo, L., Lussiana, C., Molla, P., Chatzifotis, S., 2014b. Substitution of fish meal by *Tenebrio molitor* meal in the diet of *Dicentrarchus labrax* juveniles. In:Vantomme, P., Munke, C., van Huis, A. (Eds.), 1st International Conference “Insects to Feed the World”. Wageningen University, Ede-Wageningen, The Netherlands, p.70.

Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D.J Souza, E., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the

utilization of sustainable plant products in aquafeeds: a review. Aquac Res. 38(6), 551-579.

Glencross, B.D., Booth, M., Allan, G.L., 2007. A feed is only as good as its ingredients - A review of ingredient evaluation strategies for aquaculture feeds. Aquac. Nutr. 13, 17–34.

Goering, H.K., Van Soest, P.J., 1970. Forage fiber analyses. Agricultural Handbook 379. Washington DC, USA.

Gray, J.I., 1977. Measurement of lipid oxidation: a review. J. Am. Oil Chem. Soc. 55, 539–546.

Green, J.A., Hardy, R.W., Brannon, E.L., 2004. The optimum dietary essential: nonessential amino acid ratio for rainbow trout (*Oncorhynchus mykiss*), which maximizes nitrogen retention and minimizes nitrogen excretion. Fish Physiol. Biochem. 27,109–115.

Greene, D.H.S., Selivonchick, D.P., 1990. Effects of dietary vegetable, animal and marine lipids on muscle lipid and haematology of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 89, 165–182.

Hardy, R.W., 2002. Rainbow trout, *Oncorhynchus mykiss*. In. Wrbster, C.D., Lim, C., Nutrient Requirements and Feeding of Finfish for Aquaculture. CABI. 14, 184-202.

Harnden, L.M., Tomberlin, J.K., 2016. Effects of temperature and diet on black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), development. Forensic Sci. Int. 266, 109–116.

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.

Hermansson, A.M., 1986. Water- and fat holding. In: Mitchell J.R., Ledward D.A. (eds.), Functional Properties of Food Macromolecules, Elsevier 273-314.

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

Hernandéz-Herrero, M.M., Duflos, G., Malle, P., Bouquelet, S., 2003. Collagenase activity and protein hydrolysis as related to spoilage of iced cod (*Gadus morhua*). Food Res. Int. 36, 141-147.

Honikel, K.O., 1989. The meat aspects of water and food quality. In: Hardman T.M. (ed.), Water and Food Quality, Elsevier 277-304.

Hossain, M.A., Nahar, N., Kamal, M., 1997. Nutrient digestibility coefficients of some plant and animal proteins for rohu (*Labeo rohita*). Aquaculture 151, 37–45.

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 Chem. 148, 86–91.

Hultmann, L., Rustad, T., 2002. Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). J. Aquat. Food Prod. Technol. 11, 105-123.

Hyldig, G., Nielsen, D., 2001. A review of sensory and instrumental methods used to evaluate the texture of fish muscle. J. Texture Stud. 32, 219-242.

Idowu, A.B., Amusan, A.A.S., Oyediran, A.G., 2003.The response of *Clarias gariepinus* fingerlings (Burchell1822) to the diet containing Housefly maggot (*Musca domestica*) (L.). Niger. J. Anim. Prod. 30, 139–144.

INE. 2016. Estatísticas da Pesca 2015. Instituto Nacional de Estatística, I.P., Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos (DGRM), Lisboa, Portugal.

INPN, Inventaire National du Patrimoine Naturel (https://inpn.mnhn.fr/espece/cd_nom/217341). (February, 2016).

Irie, M., Izumo, A., Mohri, S., 1996. Rapid method for determining water-holding capacity in meat using video-image analysis and simple formulae. Meat Sci. 42 (1), 95-102.

Jabir, M.D.A.R., Razak, S.A., Sabaratnam, V., 2012b. Effect of mushroom supplementation as a prebiotic compound in superworm based diet on growth performance of red tilapia fingerlings. Sains Malaysiana 41, 1197–1203.

Jabir, M.D.A.R., Razak, S.A., Vikineswary, S., 2012a. Nutritive potential and utilization of superworm (*Zophobas morio*) meal in the diet of Nile tilapia (*Oreochromis niloticus*) juvenile. Afr. J. Biotechnol. 11, 6592–6598.

Jayaram, M.G., Shetty, H.P.C., Udupa, K.S., 1980. Organoleptic evaluation of flesh of carps fed on different kinds of feeds. Mysore J. Agric. Sci. 14, 421–424.

Jeyachandran, P., Raj, S.P., 1976. Experiments with artificial feeds on *Cyprinus carpio* fingerlings. J. Inland Fish. Soc. India 8, 33–37.

Ji, H., Zhang, J.L., Huang, J.Q., Cheng, X.F., Liu, C., 2013.Effect of replacement of dietary fish meal with silkworm pupae meal on growth performance, body composition, intestinal protease activity and health status in juvenile Jian carp (*Cyprinus carpio* var Jian). Aquac. Res., 1–13 (Earlyviewonlineversion).

Ji, W., Wang, Y., Tang, J., 2010. Apparent digestibility coefficients of selected feed ingredients for Japanese seabass (*Lateolabrax japonicus*) reared in sea water. J. Fish. China 34, 101–107.

Jiang, W.D., Wu, P., Tang, R.J., Liu, Y., Kuang, S.H., Jiang, J., Tang, L., Tang, W.N., Zhang, Y.A., Zhou, X.Q., Feng, L., 2016. Nutritive values, flavor amino acids, healthcare fatty acids and flesh quality improved by manganese referring to up-regulating the antioxidant capacity and signaling molecules TOR and Nrf2 in the muscle of fish. Food Res. Int. Available in: http://dx.doi.org/10.1016/j.foodres.2016.09.020 (July 2016).

Jintasatapom, O., Chumkam, S., Jintasatapom, O., 2011. Substitution of silkworm pupae (*Bombyx mori*) for fish meal in broodstock diets for snake skingourami (*Trichogaster pectoralis*). J. Agric. Sci. Technol. A1, 1341–1344.

Johansson, L., Kiessling, A., Kiessling, K.H., Berglund, L., 2000. Effects of altered ration levels on sensory characteristics, lipid content and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). Food Qual. Prefer. 11, 247–254.

Johri, R., Singh, R., Johri, P.K., 2011. Impact of formulated plant and animal supplemented diets on nutritional efficiency, growth and body composition in juveniles of *Clarias batrachus* in experimental tanks. J. Exp. Zool. India 14, 59–68.

Jonathan Tan, U/D. https://taxo4254.wikispaces.com/Hermetia+Illucens. (February 2016).

Kaale, L.D., Eikevik, T.M., Rustad, T., Nordtvedt, T.S., 2014. Changes in water holding capacity and drip loss of Atlantic salmon (*Salmo salar*) muscle during superchilled storage. Food Sci. Technol. 55, 528-535.

Kalogeropoulos, N., Andrikopoulos, N., Hassapidou, M., 2004. Dietary evaluation of Mediterranean fish and molluscs pan-fried in virgin olive oil. J. Sci. Food Agric., 84, 1750–1758.

Kause, A., Quinton, C., Airaksinen, S., Ruohonen, K., Koskela, J., 2011. Quality and production trait genetics of farmed European whitefish, *Coregonus lavaretus*. J. Anim. Sci. 89, 959–971.

Kim, Y.K., 1974. Determination of true digestibility of dietary proteins in carp with Cr2O3 containing-diet. Nippon Suisan Gakk. 40, 651–653.

Knight, J., Rowley, A.F., 1995. Immunoregulatory activities of eicosanoids in the rainbow trout (*Oncorhynchus mykiss*). Immunology. 85, 389-393.

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.

Larsen, D., Quek, S., Eyres, L., 2011. Evaluating instrumental colour and texture of thermally treated New Zealand King salmon (*Oncorhynchus tshawytscha*) and their relation to sensory properties. Food Sci. Technol. 44, 1814-1820.

Lee, J., Choi, I.C., Kim, K.T., Cho, S.H., Yoo, J.Y., 2012. Response of dietary substitution of fish meal with various protein sources on growth, body composition and blood chemistry of olive flounder (*Paralichthys olivaceus*, Temminck & Schlegel, 1846). Fish Physiol. Biochem. 38, 735–744.

Lefevre, F., Cardinal, M., Bugeon, J., Labbe, L., Médale, F., Quillet, E., 2015. Selection for muscle fat content and triploidy affect flesh quality in pan-size rainbow trout, *Oncorhynchus mykiss*. Aquaculture 448, 569–577.

Lesiow, T., Ockerman, H.W., Dabrowski, K, 2009. Composition, properties and sensory quality of rainbow trout affected by feed formulations. J. World Aquacult. Soc., 40 (5), 678-686.

Lhuillery, C., Mebarki, S., Lecourtier, M.J., Demarne, Y., 1988. Influence of different dietary fats on the incorporation of exogenous fatty-acids into rat adipose glycerides. J. Nutr. 118 (12), 1447–1454.

Li, P., Mai, .K., Trushenski, .J., Wu, G., 2008. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. In: Springer.

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

Lindner, P., 1919. Extraction of fat from small animals. Z. Tech. Biol. 7, 213.

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, 74.

López-Luna, J., Torrent, F., Villarroel, M., 2014. Fasting up to 34 °C days in rainbow trout, *Oncorhynchus mykiss*, has little effect on flesh quality. Aquaculture 420–421, 63–70.

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 Chem. 93, 619–625.

Madu, C.T., Ufodike, E.B.C., 2003. Growth and survival of catfish (*Clarias anguillaris*) juveniles fed live tilapia and maggot as unconventional diets. J. Aquat. Sci. 18, 47–51.

Magalhães, R., Coutinho, F., Pousão-Ferreira, P., Aires, T., Oliva-Teles, A., Peres, H., 2015. Corn distiller's dried grains with solubles: Apparent digestibility and digestive enzymes activities in European seabass (*Dicentrarchus labrax*) and meagre (*Argyrosomus regius*). Aquaculture 443, 90–97.

Makkar, H.P.S., 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.

Martelli, R., Franci, O., Lupi, P., Faccenda, F., Parisi, G., 2014. Physico-chemical traits of raw and cooked fillets of rainbow trout (*Oncorhynchus mykiss*) from different strains and farms. Ital. J. Anim. Sci. 13, 693–702.

Martinez, O., Salmerón, J., Guillen, M.D., Casa, C., 2007. Textural and physiochemical changes in salmon (*Salmo salar*) treated with commercial liquid smoke flavourings. Food Chem. 100, 498-503.

Marty-Mahé, P., Loisel, P., Fauconneau, B., Haffray, P., Brossard, D., Davenel, A., 2004. Quality traits of brown trout (*Salmo trutta*) cutlets described by automated color image analysis. Aquaculture 232, 225-240.

Médale, F., Le Boucher, R., Dupont-Nivet, M., Quillet, E., Aubin, J., Panserat, S., 2013. Des aliments à base de végétaux pour les poissons d'élevage. INRA Prod. Anim. 26, 303–316.

Ming, J., Ye, J., Zhang, Y., Yang, X., Wu, C., Shao, X., Liu, P., 2013. The influence of maggot meal and l-carnitine on growth, immunity, antioxidant indices and disease resistance of black carp (*Mylopharyngodon piceus*). J. Chin. Cereals Oils Assoc. 28, 80–86.

Mørkøre, T., Hansen, A.A., Unander, E., Einen, O., 2002. Composition, liquid leakage, and mechanical properties of farmed rainbow trout: Variation between fillet sections and the impact of ice and frozen storage. J. Food Sci. 67, 1933–1938.

Mørkøre, T., Vallet, J.L., Cardinal, M., Gomez-Guillen, M.C., Montero, P., Torrisen, O.J. Nordtvedt, R., Sigurgisladottir, S., Thomassen, M.S., 2001. Fat content and fillet shape of Atlantic salmon: relevance for processing yield and quality of raw and smoked products. J. Food Sci. 66, 1348-1354.

Morrison, W.R., Smith, L.M., 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with born fluoridemethanol. J. Lipid Res. 5, 600-608.

Nandeesha, M.C., Gangadhara, B., Manissery, J.K., 1999. Silkworm pupa oil and sardine oil as an additional energy source in the diet of common carp, *Cyprinus carpio*. Asian Fish. Sci. 12, 207–215.

Nandeesha, M.C., Gangadhara, B., Varghese, T.J., Keshavanath, P., 2000. Growth response and flesh quality of common carp, *Cyprinus carpio* fed with high levels of non-defatted silkworm pupae. Asian Fish. Sci. 13, 235–242.

Nash, C.E., 2011. The history of aquaculture. 1st edition. Wiley-Blackwell, Iowa.

Newton, G.L., Booram, C.V., Barker, R.W., Hale, O.M., 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. J. Anim. Sci. 44, 395–400.

Newton, G.L., Sheppard, D.C., Burtle, G., 2008. Black soldier fly prepupae: a compelling alternative to fish meal and fish oil. Public comment on alternative feeds for aquaculture, NOAA 15/11//2007 - 29/2/2008.

Newton, G.L., Sheppard, D.C., Watson, D.W., Burtle, G.J., Dove, C.R., Tomberlin, J.K., Thelen, E.E., 2005. The black soldier fly, *Hermetia illucens* as a manure management/resource recovery tool. In: Symposium on the State of the Science of Animal Manure and Waste Management, San Antonio, TX, USA.

Ng, W.K., Liew, F.L., Ang, L.-P., Wong, K.W., 2001. Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus.* Aquac. Res. 32, 273–280.

Nishimura, T., Liu, A., Hattori, A., Takahashi, K., 1998. Changes in mechanical strength of intramuscular connective tissue during postmortem aging of beef. J. Anim. Sci. 76, 528-532.

Nowak, V., Persijn, D., Rittenschober, D., Charrondiere, U.R., 2016. Review of food composition data for edible insects. Food Chem. 193, 39–46.

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

Nsofor, C.J., Osayamwen, E.M., Ewuim,S.C., Etaga, H.O., 2008. Effects of varying levels of maggot and fishmeal on food utilization and growth of *Clarias gariepinus* fingerlings reared in net hopas in concrete ponds. Nat. Appl. Sci. J. 9, 79–84.

Obemeata, O., Nnenna, F.P., Christopher, N., 2011. Microbiological assessment of stored *Tilapia guineensis*. Afr. J. Food Sci. 5 (4), 242–247.

Ocaño-Higuera, V.M., Maeda-Martínez, A.N., Marquez-Ríos, E., Canizales-Rodríguez, D.F., Castillo-Yáñez, F.J., Ruíz-Bustos, E., Graciano-Verdugo, A.Z., Plascencia-Jatomea, M., 2011. Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. Food Chem. 125, 49–54.

Offer, G., Cousins, T., 1992. The mechanism of drip production- formation of 2 compartments of extracellular space in muscle postmortem. J. Sci. Food Agric. 58, 107-116.

Ogunji, J.O., Kloas, W., Wirth, M., Neumann, N., Pietsch, C., 2008a. Effect of housefly maggot meal (magmeal) diets on the performance, concentration of plasma glucose, cortisol and blood characteristics *of Oreochromis niloticus* fingerlings. J. Anim. Physiol. Anim. Nutr. 92, 511–518.

Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C., Rennert, B., 2008b. Housefly maggot meal (magmeal) as a protein source for *Oreochromis niloticus* (Linn.). Asian Fish. Sci. 21, 319–331.

Ogunji, J.O., Nimptsch, J., Wiegand, C., Schulz ,C., 2007. Evaluation of the influence of housefly maggot meal (magmeal) diets on catalase, glutathione S-transferase and glycogen concentration in the liver of *Oreochromis niloticus* fingerling. Comp. Biochem. Physiol. A147, 942–947.

Ogunji, J.O., Pagel, T., Schulz, C., Kloas, W., 2009. Apparent digestibility coefficient of housefly maggot meal (magmeal) for Nile tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*). Asian Fish. Sci. 22, 1095–1105.

Oliva-Teles, A., 2012. Nutrition and health of aquaculture fish. J. Fish Dis. 35, 83–108.

Olsen, R.L., Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends Food Sci. Technol. 27, 120–128.

Olsson, G.B., Ofstad, R., Lodemel, J.B., Olsen, R.L., 2003. Changes in water holding capacity of halibut muscle during cold storage. Lebensmittel-Wissenshaft und Technologie (LWT) 36, 771-778.

Omoyinmi, G.A.K., Olaoye, O.J., 2012. Growth performance of Nile tilapia *Oreochromis niloticus* fed diets containing different sources of animal protein. Lybian Agric. Res. Center J. Int. 3, 18–23.

Ossey, Y.B., Koumi, A.R., Koffi, K.M., Atse, B.C., Kouame, L.P., 2012. Use of soybean, bovine brain and maggot as sources of dietary protein in larval *Heterobranchus longifilis* (Valenciennes, 1840). J. Anim. Plant Sci. 15, 2099–2108.

Ostaszewska, T., Dabrowski, K., Kwasek, K., Verri, T., Kamaszewski, M., Sliwinski, J., Napora-Rutkowski, L., 2011. Effects of various diet formulations (experimental and commercial) on the morphology of the live rand intestine of rainbow trout (*Oncorhynchus mykiss*) juveniles. Aquac. Res. 42, 1796–1806.

Oyelese, O.A., 2007. Utilization of compounded ration and maggot in the diet of *Clarias gariepinus*. Res. J. Appl. Sci. 2, 301–306.

Pacheco-Aguilar, R., Lugo-Sanchez, M.E., Robles-Burgueno, M.R., 2000. Postmortem biochemical characteristics of Monterey sardine muscle stored at 0 °C. J. Food Sci. 65, 40-47.

Peres, H., Oliva-Teles, A., 2009. The optimum dietary essential amino acid profile for gilthead seabream (*Sparus aurata*) juveniles. Aquaculture 296(1-2), 81-86.

Piccolo, G., Marono, S., Gasco, L., Iannaccone, F., Bovera, F., Nizza, A., 2014. Use of *Tenebrio molitor* larvae meal in diets for Gilthead seabream *Sparus aurata* juveniles. In: Vantomme, P., Munke, C., van Huis, A. (Eds.), 1st International Conference "Insects to Feed the World". Wageningen University, Ede-Wageningen, The Netherlands, p.68.

Potenza, M., Sabatino, G., Giambi, F., Rosi, L., Papini, A.M., Dei, L., 2013. Analysis of egg-based model wall paintings by use of an innovative combined dot-Elisa and UPLC-based approach. Anal. Bioanal. Chem. 405, 691-701.

Purslow, P.P., Schäfer, A., Kristensen, L., Bertram, H.C., Rosenvold, K., Henckel, P., 2001. Water-holding of pork: Understanding the mechanisms. Proceedings 54th Reciprocal Meat Conference, 134-142.

Rahman, M.A., Zaher, M., Mazid, M.A., Haque, M.Z., Mahata, S.C., 1996. Replacement of costly fishmeal by silkworm pupae in diet of mirror carp (*Cyprinus carpio* L.). Pak. J. Sci. Ind. Res. 39, 64–67.

Rana, K.J., Siriwardena, S., Hasan, M.R., 2009. Impact of rising feeding resident prices on aquafeeds and aquaculture production. Fisheries and Aquaculture Technical Paper. FAO, Rome, Italy.

Rangacharyulu, P.V., Giri, S.S., Paul, B.N., Yashoda, K.P., Rao, R.J., Mahendrakar, N.S., Mohanty, S.N., Mukhopadhyay, P.K., 2003. Utilization of fermented silkworm pupae silage in feed for carps. Bioresour. Technol. 86, 29–32.

Rasmussen, R.S., Ostenfeld, T.H., Rønsholdt, B., McLean, E., 2000. Manipulation of end-product quality in rainbow trout with finishing diets. Aquaculture Nutr. 6, 17-23.

Ravi, C., Jeyashree, A., RenukaDevi, K., 2011. Antimicrobial peptides from insects: an overview. Res. Biotechnol. 2, 1–7.

Regost, C., Arzel, J., Cardinal, M., Laroche, M., Kaushik, S.J., 2001. Fat deposition and flesh quality in seawater reared triploid brown trout (*Salmo trutta*) as affected by dietary fat levels and starvation. Aquaculture 193, 325-345.

Robb, D.H.F., Kestin, S.C., Warriss, P.D., Nute, G.R., 2002. Muscle lipid content determines the eating quality of smoked and cooked Atlantic salmon (*Salmo salar*). Aquaculture 205, 345–358.

Roncarati, A., Gasco, L., Parisi, G., Terova, G., 2014a. Growth performance of common catfish (*Ameiurus melas* Raf.) fingerlings fed insect meal diets. In: Vantomme, P., Munke, C., van Huis, A. (Eds.), 1st International Conference "Insects to Feed the World". Wageningen University, Ede-Wageningen, The Netherlands, p.162.

Roncarati, A., Mordenti, O., Stocchi, L., Melotti, P., 2014b. Comparison of growth performance of common catfish *Ameiurus melas*, Rafinesque 1820, reared in pond and in recirculating aquaculture system. J. Aquac. Res. Dev. 5, 218.

Rørå, A.M.B., Kvåle, A., Mørkøre, T., Rørvik, K.A., Steien, S.H., Thomassen, M.S., 1998. Process yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*) in relation to raw material characteristics. Food Res. Int. 31, 601-609.

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. Int. J. Food Sci. Technol. 42, 342–351.

Rumpold, B.A., Schluter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. Innov. Food Sci. Emerg. Technol. 17, 1–11.

Rustad, T., 1992. Muscle chemistry and the quality of wild and farmed cod. In H.H. Huss et al. (Eds). Elsevier Science Publishers B.V.

Rustad, T., 2010. Handbook of seafood and seafood analysis. Lipid Oxidation. CRC Press, Florida. 87-93 pp.

Ruxton, C.H.S., Calder, P.C., Reed, S.C., Simpson, M.J.A., 2005. The impact of long-chain n−3 polyunsaturated fatty acids on human health. Nutr. Res. Rev. 18, 113–129.

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 Chem. 141, 2466–2472.

Salih, A.M., Smith, D.M., Price, J.F., Dawson, L.E., 1987. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poult. Sci. 66, 1483-1488.

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. Livest. Prod. Sci. 77(2), 187-194.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, USA, 181–257 pp.

Sawhney, S., 2014. Effect of partial substitution of expensive ingredient .e. fishmeal on the growth of *Tor putitora* fed practical diets. J. Int. Acad. Res. Multidiscipl. 2(7), 482–489.

Schubring, R., 2008. Comparative study of the DSC pattern, color, texture and water-binding capacity of rainbow trout muscle during heating. J. Food Process. Pres. 32, 190-218.

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 Aquacult. Soc. 42, 34–45.

Secci, G., Serra, A., Concollato, A., Conte, G., Mele, M., Olsen, E., Parisi, G., 2015. Carbon monoxide as stunning / killing method on farmed Atlantic salmon (*Salmo salar*): effects on lipid and cholesterol oxidation. J. Sci. Food Agric. 96(7), 2426-2432.

Shaviklo, G.R., Thorkelsson, G., Arason, S., 2010. The influence of additives and frozen storage on functional properties and flow behaviour of fish protein isolates from haddock (*Melanogrammus aeglefinus*) Turk. J. Fish. Aquat. Sci. 10, 333-340.

Sikorski, Z.E., Scott, D.N., Buisson, D.H., 1984. The role of collagen in the quality and processing of fish. Crit. Rev. Food Sci. Nutr. 20, 301-343.

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 anesthesia followed by chilling on ice/water slurry for potential implementation in aquaculture. Aquacult. Res. 45, 1759–1770.

Sinclair, A.J., Begg, D., Mathai, M., Weisinger, R.S., 2007. Omega 3 fatty acids and the brain: review of studies in depression. Asia Pac. J. Cli. Nutr. 16(1), 391–397.

Skipnes, D., Østby, M.L., Hendrickx, M.E., 2007. A method for characterising cook loss and water holding capacity in heat treated cod (*Gadus morhua*) muscle. J. Food Eng. 80, 1078-1085.

Skjervold, P.O., Fjæra, S.O., Øtsby, P.B., Isaksson, T., Einen, O., Taylor, R., 2001. Properties of salmon flesh from different locations on pre- and post-rigor fillets. Aquaculture 53, 279-286.

Skretting, 2016. http://www.skretting.com/it-IT/ (February, 2016).

Sogbesan, A.O., Ajuonu, N., Musa, B.O., Adewole, A.M., 2006. Harvesting techniques and evaluation of maggot meal as animal dietary protein source for *Heteroclarias* in outdoor concrete tanks. World J. Agric. Sci. 2, 394–402.

Sogbesan, A.O., Ugwumba, A.A.A., 2008. Nutritional evaluation of Termite (*Macrotermis subhyalinus*) meal as animal protein supplement in the diets of *Heterobranchus longifilis* (Valenciennes, 1840) fingerlings. Turk. J. Fish. Aquat. Sci. 8, 149–157.

Solomon, S.G., Sadiku, S.O.E., Tiamiyu, L.O., 2007. Wing reproductive termite (*Macrotremes nigeriensis*) – soybean (*Glyxine max*) meals blend as dietary protein source in the practical diets of *Heterobranchus bidorsalis* fingerlings. Pak. J. Nutr. 6, 267–270.

Srinivasan, S., Xiong, Y.L., Decker, A., 1996. Inhibition of protein and lipid oxidation in beef heart surimi-like material by anti-oxidants and combinations of pH, NaCl, and buffer type in the washing media. J. Agric. Food Chem. 144, 119-125.

Stanković, D., Crivelli, A.J., Snoj, A., 2015. Rainbow trout in Europe: introduction, naturalization, and impacts. Rev. Fish. Sci. Aquac. 23, 39–71.

STECF. 2014. The economic performance of the EU aquaculture sector (STECF 14-18). Publications Office of the European Union, Luxembourg, EUR 27033 EN, JRC 93169, 451pp.

Steffens, W., 1997. Effects of variation in essential fatty acids in fish feeds on nutritive value of fresh water fish for human. Aquaculture 151, 97-119.

Stéphan, G., Guillaume, J., Lamour, F., 1995. Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: effect of dietary vitamin E and dietary n-6 or n-3 polyunsaturated fatty acids. Aquaculture 130, 251–268.

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

Stubhaug, I., Lie, Ø., Torstensen, B.E., 2007. Fatty acid productive value and b-oxidation capacity in Atlantic salmon tissues (*Salmo salar* L.) fed on different lipid sources along the whole growth period. Aquacult. Nutr. 13, 145–155.

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. Aquacult. Eng. 63, 16–24.

Susanto, E., Agustini, T.W., Ritanto, E.P., Dewi, E.N., Swastawati, F., Undip, K., Semarang, T., 2011. Changes in oxidation and reduction potential and pH of tropical fish during storage. J. Coast. Dev.,14(3), 223–234.

Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285, 146–158.

Taylor, R.G., Fjaera, S.O., Skjervold, P.O., 2002. Salmon fillet texture is determined by myofiber-myofiber and myofiber-myocommata attachment. J. Food Sci., 67, 2067-2071.

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

Thomassen, M.S., Røsjø, C., 1989. Different fats in feed for salmon: influence on sensory parameters, growth rate and fatty acids in muscle and heart. Aquaculture 79, 129–135.

Torstensen B.E., Frøyland L., Lie Ø., 2004. Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil. Effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein composition and lipogenic enzyme activities. Aquacult. Nutr. 10, 175–192.

Tran, G., Heuzé, V., Makkar, H.P.S., 2015. Insects in fish diets. Anim. Front. 5, 37–44.

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. J. Fish. Sci. 1, 97–103.

Turchini, G.M., Gunasekera, R.M., De Silva, S.S., 2003. Effect of crude oil extracts from trout offal as a replacement for fish oil in the diets of the Australian native fish Murray cod (*Maccullochella peelii peelii*). Aquacult. Res., 34, 697–708.

Turchini, G.M., Hermon, K., Moretti, V.M., Caprino, F., Busetto, M.L., Bellagamba, F., Rankin, T., Francis, D.S., 2013. Seven fish oil substitutes over a rainbow trout grow-out cycle: II) Effects on final eating quality and a tentative estimation of feed-related production costs. Aquacult Nutr. 19, 95-109

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

United Nations, 2015. World Population Prospects The 2015 Revision. Working Paper No. ESA/P/WP.241.

van der Spiegel, M., Noordam, M.Y., van der Fels-Klerx, H.J., 2013. Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Compr. Rev. Food Sci. Food Saf. 12, 662–678.

van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., Vantomme, P., 2013. Edible insects - Future prospects for food and feed security. FAO Forestry Paper 171. Verbeke, W., Spranghers, T., De Clercq, P., De Smet, S., Sas, B., Eeckhout, M., 2015. Insects in animal feed: Acceptance and its determinants among farmers, agriculture sector stakeholders and citizens. Anim. Feed Sci. Technol. 204, 72–87.

Veland, J.O., Torrissen, O.J., 1999. The texture of Atlantic salmon (*Salmo salar*) muscle as measured instrumentally using TPA and Warner-Bratzler shear test. J. Sci. Food. Agr. 79, 1737-1746.

Venkatesh, B., Mukherji, A.P., Mukhopadhyay, P.K., Dehadrai, P.V., 1986. Growth and metabolism of the catfish *Clarias batrachus* fed with different experimental diets. Proc. Indian Acad. Sci. Anim. Sci. 95, 457–462.

Walton, M.E.M., Vilas, C., Coccia, C., Green, A.J., Cañavate, J.P., Prieto, A., van Bergeijk, S.A., Medialdea, J.M., Kennedy, H., King, J., Le Vay, L., 2015. The effect of water management on extensive aquaculture food webs in the reconstructed wetlands of the Doñana Natural Park. Aquaculture. 448, 451-463.

Wang, B., Liu, Y., Feng, L., Jiang, W., Kuang, S., Jiang, J., 2015. Effects of dietary arginine supplementation on growth performance, flesh quality, muscle antioxidant capacity and antioxidant-related signalling molecule expression in young grass carp (*Ctenopharyngodon idella*). Food Chem. 167, 91–99.

Zhang, J.B., Zheng, L.Y., Liu, X.L., Yu, Z.N., 2014. Kitchen waste converted by black soldier fly and partly substituting soymeal in chicken feed. In: Abstract Book Conference Insects to Feed The World, The Netherlands, 14–17 May, 152.

Appendix

Appendix 1. Published studies performed with several fish species using insects of different orders in order to fishmeal replacement. (Modified from Henry et al., 2015)

Insect	Fish	% CP in CD	% FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	Similar WG	Reduced WG	
Orthoptera								
Z. variegatus	African catfish (*Clarias garieinus*)	31	30	50		**13**; 16; 25% (**25**; 50; 75% FMr)	35% (100% FMr)	(Alegbeleye et al., 2012)
P. pictus	Walking catfish (*Clarias batrachus*)	n.a	n.a	40			(100% FMr)	(Johri et al., 2011)
L. migratoria	Nile tilapia juvenile (*Oreochromis niloticus*)	n.a.	n.a.	30–35		25% FMr		(Emehinaiye, 2012)
Isoptera								
Macrotermes	Vundu catfish (*Heterobranchus longifilis*)	30	43	45		7.5; 15% (25; 50% FMr)	22.5, 30% (75; 100% FMr)	(Sogbesan and Ugwumba, 2008)
Macrotermes	African catfish (*Clarias garieinus*)	56.6	39	50	**5.8%** (75% SBMr)	4.6–8.5% (0–100% SBMr)		(Solomon et al., 2007)
Coleoptera								
T. molitor	African catfish (*Clarias garieinus*)	n.a	34	40–43		**50% larvae (am) + 50% pellets (pm)**; 100% larvae		(Ng et al.,2001)

Appendix 1 (Continued)

Insect	Fish	% CP in CD	% FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	Similar WG	Reduced WG	
T. molitor	African catfish (*Clarias garieinus*)	35	40	40-50	**9% (20%FMr)**	17; 26% (40; 60% FMr)	35; 43% (80; 100% FMr)	(Ng et al., 2001)
T. molitor	Common catfish (*Ameiurus melas*)	50	51	40			50% (100% FMr)	(Roncarati et al., 2014a, 2014b)
T. molitor	Gilthead seabream (*Sparus aurata*)	50	44	40-42		12.5 (25% FMr)	25 (50% FMr)	(Piccolo et al., 2014)
T. molitor	European seabass (*Dicentrarchus labrax*)	70	55	42-48		17.5 (25% FMr)	35 (50% FMr)	(Gasco et al., 2014b)
T. molitor	Rainbow trout (*Oncorhynchus mykiss*)	75	45	42		19; 38 (25; 50% FMr)		(Gasco et al., 2014a)
O. rhinoceros	African catfish (*Clarias garieinus*)	50	39	50		**14**; 33; 57; 80% (**16**; 35; 62; 100% FMr)		(Fakayode and Ugwumba, 2013)
O. rhinoceros	Vundu catfish (*Heterobranchus longifilis*)	50	39	45		**14**; 33; 57; 80% (**16**; 35; 62; 100% FMr)		(Fakayode and Ugwumba, 2013)

Appendix 1 (Continued)

Insect	Fish	% CP in CD	% FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	Similar WG	Reduced WG	
O. rhinoceros	Nile tilapia (*Oreochromis niloticus*)	49	35	28-30			59% (100% FMr)	(Omoyinmi and Olaoye, 2012)
Z. morio	Nile tilapia (*Oreochromis niloticus*)	30	34	30-35		**7.5**; **15**; 22.5%(**25**; **50**; 75%FMr)	30% (100% FMr)	(Jabir et al., 2012a)
Z. morio + mushroom	Nile tilapia (*Oreochromis niloticus*)	15	33	30-35		Z. morio + 0.5, **1**, 1.5% mushroom		(Jabir et al., 2012b)
Lepidoptera								
B. mory	Rohu (*Labeo rohita*)	54	35	25-30	8, **15**, 26, 43% SWP (25, **50**, 75, 100% FMr)			(Begun et al.,1994)
B. mory	Rohu (*Labeo rohita*)	67	34	35–40	**50% non-defatted**; 42% defatted (100%FMr)			(Hossain et al.,1997)
B. mory	Common carp (*Cyprinus carpio*)	25	n.a.	30–38		21% (100% FMr)		(Kim, 1974) (in Japanese)

Appendix 1 (Continued)

Insect	Fish	% CP in CD	% FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	Similar WG	Reduced WG	
B. mory	Common carp (*Cyprinus carpio*)	25	30	30–38		Basal diet + 3;6; **9**% SWP oil		(Nandeesha et al.,1999)
B. mory	Common carp (*Cyprinus carpio*)	25	28	30-38		30;40; **50**% (100% FMr)		(Nandeesha et al., 2000)
B. mory	Common carp (*Cyprinus carpio*)	11	21	30–38	**100%**			(Jeyachandran and Raj,1976)
B. mory	Common carp (*Cyprinus carpio*)	10	37	30–38		5.7% (50%FMr)	6.8-9% (60-80% FMr)	(Ji et al.,2013)
B. mory	Common carp (*Cyprinus carpio*)	n.a.	38	30–38	**30; 40%**	20%		(Rahman et al.,1996)
B. mory	Polyculture cyprinids	33	n.a.	25–40		**30**% (**100**%FMr)		(Jayaram et al.,1980)
B. mory	Polyculture cyprinids	4.5	31	25–40	**7**% ensiled SWP(**100**%FMr)	6% normalSWP (100%FMr)		(Rangacharyulu et al., 2003)
B. mory	Putitor mahseer (*Tor putitora*)	50	36	45–50		**25**% (**70**% FMr)		(Sawhney, 2014)

Appendix 1 (Continued)

Insect	Fish	% CP in CD	% FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	Similar WG	Reduced WG	
B. mory	Walking catfish (*Clarias batrachus*)	60	25	40		**58**% (**100**% FMr)		(Venkatesh et al.,1986)
B. mory	Nile tilapia (*Oreochromis niloticus*)	5	32	30–50			5%	(Boscolo et al., 2001)
B. mory	Snakeskin gourami (*Trichogaster pectoralis*)	24	32	40		15% (50% FMr)	22-29% (75, 100% FMr)	(Jintasatapom et al., 2011)
B. mory	Japanese seabasss (Lateolabrax japonicus)	n.a.	38	41		< 73% (100% FMr)		(Ji et al., 2010)
B. mory	Rainbow trout (*Oncorhynchus mykiss*)	50	40	43	**50** (**100**% FMr)			(Dheke and Gubhaju, 2013)
B. mory	Chum salmon (Oncorhynchus keta)	60	55	45		5% (7.2% FMr)		(Akiyama et al.,1984)

Appendix 1 (Continued)

Insect	Fish	% CP in CD	% FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	Similar WG	Reduced WG	
B. mory	Olive flounder (Paralichthys olivaceus)	60	55	45		**6**; 12% (**10**; 20% FMr)		(Lee et al., 2012)

Abbreviations: am: morning meal; CD: control diet; CP: crude protein; FM: fishmeal; %FMr: percentage of fishmeal replacement; n.a.: not available; pm: afternoon meal; SBMr: percentage of soyabean meal replacement; SWP: domesticated silkworm (*B. mori*); WG: weight gain.

In bold are the optimal dietary inclusion levels giving the highest fish WG.

Appendix 2. Published studies performed with several fish species using insects from Diptera order in order to fishmeal replacement. (Modified from Henry et al., 2015)

Insect	Fish	%CP in CD	%FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	WG similar	Reduced WG	
Culex pipiens								
	Rainbow trout (*Oncorhynchus mykiss*)	12.44	46	43-50			100% (66% CP)	Ostaszewska et al., 2011
Hermetia illucens								
	*Channel catfish (*Ictalurus punctatus)* + Blue tilapia (*Oreochromis aureus*)	Low(LP) or high (HP) protein	30 or 45	36–56		50% HP + 50% BSF; 25% LP + 75 % BSF; 100% BSF		Bondari and Sheppard, 1981
	Channel catfish (*Ictalurus punctatus*)	n.a.	**37.5**	25–36			100%	Bondari and Sheppard, 1987 (trial 2)
	Channel catfish (*Ictalurus punctatus*)	10	30	25–36			Basal diet + 10% BSF(100% FMr)	Bondari and Sheppard, 1987 (trial 1)
	Channel catfish (*Ictalurus punctatus*)	**25**	n.a.	25–55		6% (25% FMr)	12; 19; 25% (50; 75; 100% FMr)	Newton et al., 2005

Appendix 2 (Continued)

Insect	Fish	%CP in CD	%FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	WG similar	Reduced WG	
	Blue tilapia (*Oreochromis aureus*)	n.a.	37,5	56			100%	Bondari and Sheppard, 1987 (trial 3)
	Rainbow trout (*Oncorhynchus mykiss*)	**29**	**46**	42			16–33% (25–50% FMr)	Sealey et al., 2011
	Rainbow trout (*Oncorhynchus mykiss*)	**29**	**46**	42		18–36% (25–50% FMr)		Sealey et al., 2011
	Rainbow trout (*Oncorhynchus mykiss*)	**36**	**39**	42		15% (25% FMr)	30% (50% FMr)	St-Hilaire et al., 2007b
	Atlantic salmon (*Salmon salar*)	20	46	42–48		5; 10; 25% (25; 50; 100% FMr)		Lock et al., 2014
	Atlantic salmon (*Salmon salar*)	**20**	**46**	42–48			5; 25% (25–100% FMr)	Lock et al., 2014
	Turbot (*Psetta maxima*)	69	55	50			17; 33; 49; 64; 76% (18; 36; 52; 69; 82% FMr)	Kroeckel et al., 2012

Appendix 2 (Continued)

Insect	Fish	%CP in CD	%FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	WG similar	Reduced WG	
Musca domestica								
	Black carp (*Mylopharyngodon piceus*)	n.a.	n.a.	40	Basal diet + **2.5**%			Ming et al., 2013
	Gibel carp (*Carassius auratus*)	**39**	38	40		**39**% (**71**% FMr)		Dong et al., 2013
	Mudfish (*Clarias anguillaris*)	0	n.a.	40	100%			Achionye-Nzeh and Ngwudo, 2003
	Vundu catfish (*Heterobranchus longifilis*)	0	35	45		81%		Ossey et al., 2012
	African catfish (*Clarias garieinus*)	n.a.	n.a.	40–55		12.5; 25% (50, 100% FMr)		Nsofor et al., 2008
	African catfish (*Clarias garieinus*)	**0**	n.a.	40–55		12.5; 50%	100% inclusion	Idowu et al., 2003
	African catfish (*Clarias garieinus*)	25	41	40–43		**12.5**–**25**% (**50**–**100**% FMr)		Aniebo et al., 2009
	African catfish (*Clarias garieinus*)	25	n.a.	50		12.5; 25% (50; 100% FMr)		Aniebo et al., 2011

Appendix 2 (Continued)

Insect	Fish	%CP in CD	%FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	WG similar	Reduced WG	
	African catfish (*Clarias garieinus*)	**25**	**40**	50		27% defatted oven / 32% sun-dried (100% FMr)	33.5% oven-dried; 35% sun-dried (100% FMr)	Fasakin et al., 2003
	African catfish (*Clarias garieinus*)	3.5	35	40–43	50% pellets + **50**% maggots		25% pellets + 75% maggots; 100% maggots	Oyelese, 2007
	African catfish (*Clarias garieinus*)	n.a.	40	40–43	50% pellets + 50% maggots; 100% maggots			Madu and Ufodike, 2003
	African catfish (*Clarias garieinus*)	**30**	**36**	50		7.5–15% (25; 50% FMr)	22.5–30% (75; 100% FMr)	Adewolu et al., 2010
	Hybrid catfish (*Heteroclarias*)	30	40	45–50	7.5% (25% FMr)	15; 22.5% (50; 75 FMr%)	30% (100% FMr)	Sogbesan et al., 2006
	Dark barbel catfish (*Pelteobagrus vachelli*)	**39.4**	**38**	40–45			39% (71% FMr)	Dong et al., 2013
	Nile tilapia (*Oreochromis niloticus*)	49	35	30–35		**56**% **(100**% FMr)		Omoyinmi and Olaoye, 2012

Appendix 2 (Continued)

Insect	Fish	%CP in CD	%FM in CD	Protein requirement	% Dietary inclusion (corresponding %FM replacement)			References
					Increased WG	WG similar	Reduced WG	
	Nile tilapia (*Oreochromis niloticus*)	0	n.a.	28–50	Basal diet + 20% larvae			Ebenso and Udo, 2003
	Nile tilapia (*Oreochromis niloticus*)	n.a.	30	30–35	(50% FMr)	(25; 75; 100% FMr)		Ajani et al., 2004
	Nile tilapia (*Oreochromis niloticus*)	**52**	**36**	30–35			15; 30% (18; 37% FMr)	Ogunji et al., 2008c
	Nile tilapia (*Oreochromis niloticus*)	43	38	30–35		**15**; **25**; **35**; **45**; 55; 68%		Ogunji et al., 2007; 2008a; 2008b
	Common carp + Nile tilapia (*Oreochromis niloticus)*	30	43	28–38		(**20**; **35**; **50**; **63**; 75; 100% FMr)		Ogunji et al., 2009
	Rainbow trout (*Oncorhynchus mykiss*)	**36**	**39**	42			9.2% (25% FMr)	St-Hilaire et al., 2007a

Abbreviations: am: morning meal; CD: control diet; CP: crude protein; FM: fishmeal; %FMr: percentage of fishmeal replacement; n.a.: not available; pm: afternoon meal; SBMr: percentage of soyabean meal replacement; SWP: domesticated silkworm (*B. mori*); WG: weight gain.

In bold are the optimal dietary inclusion levels giving the highest fish WG.

* Polyculture of Channel catfish and Blue tilapia in the same tanks.