Potential of pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute: effect on growth performance and digestibility in European sea bass (*Dicentrarchus labrax)*

MASTER THESIS

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ABSTRACT

In the present study a 62-day feeding trial was carried out in recirculating aquaculture system (RAS) by replacing fish meal protein subsequently by *Hermetia illucens* meal (HM), in practical diets for European sea bass (*Dicentrarchus labrax* L. 1758) to examine the effect on growth and nutrient digestibility. A control diet without insect meal and three experimental diets were formulated with an inclusion level of 6.5 %, 13% and 19.5% of HM. The diets were fed to triplicate groups of sea bass with an initial weight of 49.50 ± 0.5 g twice a day, six days per week, by hand until apparent satiation. The different replacing level of fish meal to insect meal in the three experimental diets did not affect to growth parameters (WG, FI, FCR, FE, SGR and DGI). The apparent digestibility coefficients (ADC) between groups were not affected by the inclusion of insect meal in diets until 19.5%. In general, our study shows that the incorporation of HM protein in European sea bass diets is possible as an alternative protein resource instead of the fish meal, more expensive and less sustainable. Further researches on HM meal processing to increase nutrient utilization are needed.

Key words: European sea bass, *Dicentrarchus labrax*, fish meal, replacement, insect meal, *Hermetia illucens*, growth, digestibility.

RESUMEN

En el presente trabajo se llevó a cabo un ensayo de nutrición durante 62 días en un sistema de recirculación acuícola (RAS), en dietas para lubina (*Dicentrarchus labrax* L. 1758) con sustitución proteíca de harina de pescado por harina de insecto (HM), de la especie *Hermetia illucens,* para examinar los efectos sobre el crecimiento y digestibilidad de nutrientes. Se formularon una dieta control sin harina de insecto y otras tres dietas experimentales con un nivel de inclusión de 6,5%, 13% y 19% de harina de insecto. Cada grupo por triplicado, con un peso inicial medio por pez de 49, 50 ± 0,5 g, fueron alimentados a mano dos veces al día, seis días por semana, hasta saciedad. El nivel de sustitución de harina de pescado por harina de insecto en las tres dietas experimentales no afectó a los parámetros de crecimiento (WG, FI, FCR, FE, SGR y DGI). Los coeficientes de digestibilidad aparente (ADC) entre los diferentes grupos tampoco fueron afectados por la inclusión de harina de insecto en dietas hasta 19,5%. En general, nuestro estudio demuestra que la incorporación en dietas para lubinas de harina de insecto es posible como fuente de proteína alternativa al uso de harina de pescado, menos sostenible. Se deben llevar a cabo más investigaciones sobre el adecuado procesamiento de la harina de insecto para poder aumentar los niveles de utilización de nutrientes.

Palabras clave: lubina, *Dicentrarchus labrax*, harina de pescado, sustitución, harina de insecto, *Hermetia illucens*, crecimiento, digestibilidad.

MAIN OBJECTIVES

- Evaluation of the potential of insect meal inclusion in different levels in diets for European sea bass juveniles on: Growth performance, voluntary feed intake, feed utilization (including protein, lipid and energy budgets).
- To measure the digestibility coefficients for juvenile of European sea bass fed diets with different levels of fish meal substitution by insect meal, in order to adequate the optimum level of fish meal replacement.

1. INTRODUCTION

The global population is increasing and in order to maintain at least the current level of per capita consumption, the world will require an additional 23 millions tonnes of aquatic food by 2030, which must come from aquaculture. Although the discussion on the availability and use of aquafeed ingredients often focused on fishmeal and fish oil resources (including trash fish), considering the past trends and future predictions, sustainability of the aquaculture sector is more likely to be closely linked with the sustained supply of terrestrial animal and plant proteins, oils and carbohydrate sources for aquafeeds. On these grounds, the tremendous challenge of feeding our planet while safeguarding its natural resources for future generations has gained increasing importance in recent decades (FAO, 2014).

Intensive aquaculture production of fish, particularly carnivorous fish, is still base on high quality fish meal and fish oil. The steady decline in catches of wild fish and the increased demands for livestock and aquaculture feeds have resulted in a rapid decrease in the availability of fish meal (FM) and fish oil (FO) and in their concurrent price increase (FAO, 2014). Nevertheless, the use of these commodities aquafeeds has been decreasing (Karapanagiotidis, 2014), due to a higher demand of fisheries products, world limited availability, market supply fluctuations and raising prices, which have stimulated research on the use of more sustainable alternative feedstuffs (Gatlin *et al.*, 2007).

For carnivorous fish diets protein is the largest component, the optimal dietary levels vary with species. It is known that European sea bass juveniles have a high dietary protein requirement, ranquing from 45% to 50% (Peres and Oliva-Teles, 2006). For this reason a search of a sustainable ingredient for substituting the abusive use of fish meal, is nowadays a challenge for the nutrition area in aquaculture, because the cost of aquaculture feeds represents 40-70% of the cost of the fish produced (Rana *et al*., 2009; Wilson, 2002) and is a limited resource. So, development and use of alternatives to fish meal and fish oil in aquaculture diets continue a high priority (Glencross *et al*., 2007) in order to aquaculture production would allow to remain economically and environmentally sustainable over the long term (Barroso *et al*., 2014).

In the last decade, the use of vegetable protein rich ingredients in practical diets of carnivorous fish species, has focused much attention in order to wider the amount and diversity of plant ingredients of aqua feeds (Messina *et al*., 2013). Soya and other terrestrial plants rich in proteins and lipids have been introduced into diet of aquaculture fish to replace FM and FO (Hardy, 2002; Espe *et al*., 2006; Gatlin *et al*., 2007). However, the presence of anti-nutritional factors in plant meals (Tacon, 1993; Francis *et al*., 2001; Ogunji, 2004; Collins, 2014), the potential problems of the inflammation of the digestive tract (Merrifield *et al*., 2011) and the decreased palatability of the meal (Papatyphon and Soares, 2001) are of concern. Also, plant feedstuffs have lower protein contents and essential amino acids (EAA) imbalances, and they are often deficient in Lys and Met. Such deficiencies can primarily lead to an increase in feed intake (FI), because the fish eat more to cover its basics needs.

In this context, insects are part of the natural diet of both freshwater and marine fish (Howe *et al*., 2014; Whitley and Bollens, 2014), and in general, edible insects were found to be good sources of proteins, fat, energy, vitamins and minerals (Rumpold, 2013). In comparison to conventional livestock in general, insects have a higher feed conversion efficiency, need less amount of feed for the production of 1 kg biomass, have a higher fecundity (Nakagaki and Defoliart, 1991) and leave a small ecological footprint (no needs for arable land, low need for energy and water) (Oonincx and de Boer, 2012). It has to be investigated more in detail whether insects have the potential to replace or at least decrease the amount of fish meal and fish oil in fish feed considering the lack of polyunsaturated omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the fatty acid profiles of insects. Nowadays the studies of the replacement of fishmeal with insects in the diet of fish have emerged and the promising results have encouraged further research. In general, the AA patterns of insects are taxon-dependent (Barroso *et al*., 2014), most of insects tested in fish diets, show a good correlation with fish requirement values (Hasan, 2001; NRC, 2011;

Alegbeleye *et al*., 2012) and, in some cases, even exceed these requirements (like the yellow mealworm, *Tenebrio molitor*) (Barroso *et al*., 2014).

Another important thing to count with in aquafeeds, is the level of lipids because of the role that the play in almost every physiological process in the fish, specially growth. The energy requirements in fish are lower than those of mammals (Finke, 2002). Lipids provide essential fatty acids and energy, something essential for the effective utilization of dietary proteins. The lipid level in fish meal (8.2%) and soya meal (3.0%) is lower than that of insects, in which it usually ranges from 10 to 30% even though it is extremely variable (DeFoliart, 1991). The diet of insects is mainly responsible for the variations in the lipids and fatty acids (FAs) composition of the insects (Barroso *et al*., 2014).

Concerning the content of micronutrients of insects, it can generally be stated that the majority of insects show high amounts of potassium, calcium, iron, magnesium , selenium (DeFoliart, 1992; Finke, 2002) and also several vitamins (Schabel, 2010).

In this trial the insect species used was the black soldier fly (*Hermetia illucens,* Linneaus 1758), a fly (Diptera) that belongs to *Stratiomyidae* family. It is native from the tropical, subtropical and warm temperate regions of America. It is now widespread in tropical and warmer temparate regions between about 45ºS (Diener *et al*., 2012).

The adult fly is black and the maximum size is 15-20 mm long (Hardouin *et al*., 2003). The larvae can reach up to 27 mm in length and 6 mm in width and weigh up to 220 mg in their last larval stage (Makkar *et al*., 2014). Under ideal conditions, larvae become mature in 2 months, but the larval stage can last up to 4 months when not enough feed is available (Hardouin and Mahoux, 2003), also the duration of the pupal stage is about 14 days but can be extremely variable and last up to 5 months (Hardouin and Mahoux, 2003). The adults do not feed and rely on the fats stored from the larval stage (Diclaro and Kaufman, 2009), this is another advantage of *Hermetia illucens* over other insect species used for biomass production is that the adult does not feed and therefore does not require particular care and is not a potential carrier of diseases (Makkar *et al*., 2014).

The larvae are a high-value feed source, rich in protein and fat that contain about 40-44% protein (dry matter basis) and the amount of lipids and its fatty acid composition is extremely variable and depends on the type and lipid level of insect diet.

Larvae of Hermetia fly feed on animal manure and plant material and are capable to convert low valued organic waste into protein rich biomass (Diener *et al*., 2009). The larvae in dense population can convert large volumes of organic waste into valuable biomass (van Huis *et al*., 2013), so this can be used to solve some environmental problems associated with manure and other organic wastes from farms or processing industries. The larvae are sold for pets and fish bait, and they can be easily dried for longer storage (Leclercq, 1997; Veldkamp *et al*., 2012). Due to the amount of protein (476 g kg^{-1}, dry matter DM) and fat (118 g kg^{-1}, DM) and the well balanced essential amino acid (EAA) profile, the pre-pupae meal might be a suitable alternative for fish meal. In addition it has been suggested that the larvae contain natural antibiotics (Newton *et al*., 2008).

Studies done using this insect as fish meal substitution with Atlantic salmon (*Salmo salar)* by Lock *et al*.(2014) obtained an increased in feed conversion efficiency in diets with black soldier meal (25, 50 and 100%). In rainbow trout (*Oncorhynchus mykiss),* fish fed with diets with 25% of fish meal substitution by black soldier meal showed any significant differences between the control diet and the experimental group (St-Hilaire *et al.,* 2007).

All that factors were determinant for considerate the *Hemetia illucens* larvae as a meal in aquaculture feed, and open additional marketing opportunities for farmers as some customers are opposed to the use of fishmeal in aquaculture feeds (Tiu, 2012).

Other studies were done with different species of insects, Nandeesha *et al*. (1990) measured the growth of common carp (*Cyprinus carpio*) on diets containing variable percentages of non-defatted silkworm (*Bombyx mori*) pupae (41% crude protein) and fish meal (68% crude protein). The diet with 30% silkworm plus 0% fish meal stimulated the most growth of juvenile carp over the test period. The authors concluded that non defatted silkworm pupae could replace fishmeal in diet formulations as feed for common carp, especially because the texture, flavour, odour, and colour of this fish does not change with a change from the standard fish meal diet (Nandeesha *et al*., 1990). In a companion study, Nandeesha *et al*. (2000) increased the percentage of silkworm pupae in experimental diets. The formulated diets containing inclusion of 30%, 40%, or 50% silkworm pupae without any fish meal; the control diet consisted of fish meal without any silkworm. The authors discovered no changes in weight gain, food conversion ratio, or protein conversion ratio when they fed carp juveniles any percentage of silkworm-based diet or the control (fish meal). However, carp retained increasing amounts of protein in tissues with an increase (30–50%) in the percentage of silkworm in the diets. Carp fed the 50% silkworm diet contained more protein (but less fat) than those fed lower percentages of silkworm. Apparently, carp diets can contain up to 50% silkworm pupae without changing the growth and quality of the product (Nandeesha *et al*., 2000).

The superworm (*Zophobas morio*) is also a common insect in pet stores. One study formulated diets using protein from superworm to replace from 0% to 100% of the protein from fish meal as feed for the Nile tilapia *O. niloticus* (Jabir *et al*., 2012a). At the conclusion, they found that fish fed the diets in which protein from superworm replaced 25% or 50% of the fish meal experienced the greatest weight gain, specific growth rates, feed conversion ratio, and protein efficiency ratio.

Another specie used as insect meal is the mealworm (*Tenebrio molitor*) in 2014 Piccolo *et al*., used diets containing mealworm meal replacing 25 or 50% of fish meal protein for gilthead sea brean (*Sparus aurata)* with not affect in weight gain, but with a growth reduction and less feed conversion ratio in the fish fed with diets of 50% substitution. With the same insect meal Gasco *et al.* (2014) in European sea bass, including up to 25% of mealworm meal as a replacement of fish meal had no adverse effects on weight gain, but at 50% of inclusion results for specific growth rate and feed consumption were reduced than the fish fed with the control diet without mealworm meal.

In Mediterranean aquaculture one of the most important specie and highly commercial value in Europe of fish is the European sea bass (*Dicentrarchus labrax*), both as aquaculture resource in the Mediterranean Sea and as a seasonal catch and trophy fish in the north-eastern Atlantic Ocean. The specie belongs to the family of Moronidae. Is a specie both eurythermal and euryhaline, tolerating a great range of temperature from 2 – 32ºC in adults (Barnabe, 1990) and salinities (it possible to find the specie in fresh water and hypersalines) (Jensen *et al*., 1998).

Sea bass are carnivorous predators, feeding opportunistically on almost any available food item. The main part of the diet consists of crustaceans and fish that move on the sea bed or in the water column. Sex is determined by both genetic and environmental factors, with warmer temperatures favouring males, for this reason in the Mediterranean aquaculture, due to the warmer temperatures, the majority (75%-100%) of the adults are males (Piferrer *et al*., 2005), a condition which is usually not desired, because females are generally larger than males of the same age. Sexual maturity is reached between 2 and 4 years in the Mediterranean Sea, and between 4 and 7 years for males and 5 and 8 years for females in the Atlantic Ocean (Fishbase, 2015).

The combination of slow growth, late maturity and spawning aggregation, make sea bass particularly vulnerable to overexploitation and localized depletion. Intensive aquaculture of sea bass is mainly based on sea-cages but some land-based recirculation systems also exist in northern Europe until they are fattened to commercial size of 300-500g in 1.5 to 2 years.

In this context, the aim of the present study is to investigate and compare the effects on growth and digestibility in European sea bass (*Dicentrarchus labrax*) of the inclusion of insect meal in different levels in diets.

2. MATHERIALS AND METHODS:

2.1. Test diets

Four experimental diets, whose composition is shown in Table 1, were formulated to be isolipidic and isoproteic and the optimal levels for this species were chosen based on the figures suggested as optimal by Peres and Oliva-Teles (1999, 2002). All diets included the same level of vegetable protein inclusion, just different protein replacement percentage where done in order to have three experimental diets increasing levels of protein from insect meals. The control diet (HM0) was chosen based on the figures suggested as optimal for this specie. The experimental diets were respectively obtained from the preparation of the control diet by including 6.5, 13 and 19.5% of insect meal (corresponding to 7.5, 15, 22.5% of the total dietary protein) in substitution of fish meal. As shown in Table 1 the diets included vegetables proteins as close as possible the dietary ideal protein needs of the European sea bass (Tibaldi *et al.*, 1996). Diets were not supplemented with essential amino acids because the essential aminoacids profile was evaluated to be equal or higher than that estimated by Kaushik (1998) for European sea bass.

Table 1. Composition and proximate analysis of the experimental diets.

	HM 0	HM 6.5	HM 13	HM 19.5
Ingredients (g 100 g^{-1} diet)				
Fish meal [1]	32.40	27.50	22.70	17.80
Insect meal[2]	0.00	6.50	12.97	19.50
Corn gluten[3]	10.00	10.00	10.00	10.00
Soybean meal[4]	12.00	12.00	12.00	12.00
Wheat gluten [5]	7.10	7.40	7.64	7.90
Wheat meal[6]	17.90	15.70	13.57	11.40
Fish oil[7]	12.90	13.20	13.45	13.70
Vitamin premix[8]	1.00	1.00	1.00	1.00
Choline chloride (50%)	0.50	0.50	0.50	0.50
Mineral premix[9]	1.00	1.00	1.00	1.00
Binder[10]	1.00	1.00	1.00	1.00
Agar	1.00	1.00	1.00	1.00
Chromic oxide (Cr_2O_3)	0.50	0.50	0.50	0.50
Taurine	0.20	0.20	0.20	0.20
NaH_2PO_4	0.00	0.40	0.80	1.20
Cellulose	2.50	2.10	1.70	1.30
Proximate analyses (% dry weight)				
Dry matter (%)	91.51	93.28	94.00	92.21
Crude Protein	48.06	48.85	50.37	52.83
Crude Lipid	17.53	17.13	17.35	17.89
Ash	9.19	10.45	9.61	9.49
Grosse energy (kJ.g^{-1})	22.82	23.06	23.05	23.08
Chromic oxide	0.43	0.46	0.50	0.50

[1] Fish meal, Pesquera Centinela, Steam Dried LT, Chile (CP: 69.6%; CL 11.3%). Sorgal, S.A. Ovar, Portugal
[2] Insect meal, (CP: 52.0%; CL: 5.5%), Hermetia Deutchland GmvH & Co KG. Baruth/Mark, Germany.
[3] Corn gluten (CP: 78.6%; CL: 4.1%), Sorgal, S.A. Ovar, Portugal
[4] Soybean meal (CP: 55.1%; CL: 2.5%), Sorgal, S.A. Ovar, Portugal
[5] Wheat gluten (CP: 85.6%; CL: 1.74%), Sorgal, S.A. Ovar, Portugal
[6] Wheat meal (CP: 9.9%; CL: 3.2%), Sorgal, S.A. Ovar, Portugal.
[7] Fish oil, Sorgal, S.A. Ovar, Portugal.
[8] Vitamins (mg kg^{-1} diet): retinol, 18000 (IU kg^{-1} diet); calciferol, 2000 (IU kg^{-1} diet); alpha tocopherol, 35; menadion sodium bis., 10; thiamin, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400.
[9] Minerals (mg kg^{-1} diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dicalcium phosphate, 8.02 (g kg^{-1} diet); potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.4 (g kg^{-1} diet).
[10] Aquacube. Agil, UK.

The proximate amino acid profile of the four experimental diets is shown in Table 2. The diets were manufactured at the facilities of the Marine Zoological Station, Faculty of Science, University of Porto. All the ingredients used were ground before final mixing and steam pelleted using a laboratory pellet mill (California Pellet Mill, Crawfordsville, IN, USA) through 3 mm die.

Table 2. Estimated composition of amino acid profile of the diets.

	HM0	HM6.5	HM13	HM19.5
Arg (%)	6.5	6.4	6.3	6.3
Hys (%)	2.3	2.4	2.5	2.5
Ile (%)	4.5	4.5	4.5	4.5
Leu (%)	9.2	9.2	9.2	9.2
Lys (%)	6.2	6.0	5.9	5.7
Met (%)	2.3	2.3	2.2	2.1
Cys (%)	1.4	1.4	1.4	1.4
Phe (%)	4.9	4.9	4.8	4.8
Tyr (%)	3.8	3.5	3.3	3.0
Tre (%)	4.0	4.0	3.9	3.9
Trp (%)	1.0	0.9	0.9	0.8
Val (%)	5.2	5.3	5.4	5.5
Met+Cys (%)	3.7	3.7	3.6	3.5
Phe+Tyr (%)	8.7	8.1	7.6	7.0

For the digestibility trial, all diets were prepared and supplemented with chromic oxide, the most commonly used marker, as inert marker. After drying the pellets in a drying oven at 55ºC for 24 hours, the feeds were stored at -20ºC until use. The proximate composition of the insect meal used (*Hermetia illucens)* is shown in the next Table 3.

Table 3. Calculated analysis composition of the insect meal used.

	Dry matter (%)	Ash (%)	Lipids (%)	Protein (%)
Hermetia illucens meal (HM)	94.58	8.48	5.47	55.76

2.2. Fish and experimental conditions

The digestibility trials were performed at the Marine Zoological Station, Faculty of Science, University of Porto, with the specie European sea bass (*Dicentrarchus labrax*) juveniles provided by (*MARESA*. Mariscos de Estero S.A., Finca El Tambujal s/n, Apdo de correo 82. Ayamonte, *Huelva*).

The experimental design system consisted of a thermo-regulated recirculation water system equipped with twelve fiberglass tanks of 60 litres water capacity, each one with a faces settling column connected to the outlet designed according Cho *et al* (1982), provided with a thermostatic control and regulation of water temperature. The effluent of each tank flowed into a common drainage channel which led to the biological filter composed of two square tanks of 500 l capacity full filled with bio-balls and mechanical sand-filter. During the trial, the system ensured nearly constant and optimal water quality to fishes: water-flow was established at about 4´5 L/min per each tank, constant water temperature 26ºC, salinity 36 g l^{-1} and dissolved oxygen was kept above 80% of saturation. The experimental fish were subjected to a 12h light/12h dark artificial photoperiod regime.

One hundred and twenty European sea bass were used. Ten fishes with an average weight per fish of 49.4 ± 0.5 g were established in each tank with an initial density of 0.499 kg m^{-3}. The diets were randomly assigned to triplicate groups of fish like the next Table 4 show.

Table 4. Assignation of tank for each experimental diet.

DIET	TANK	TANK	TANK
HM 0	2	4	9
HM 6.5	3	6	8
HM 13	10	11	12
HM 19.5	1	5	7

After one week to acclimate to the experimental conditions the fishes were hand-fed until they start to refused the pellets, twice a day with the correspondent experimental diets per each tank during morning and afternoon, with minimum 6 hour between each fed, 6 days per week, over a period of 62 days. Thirty minutes after the afternoon fed, each tank and settling columns where thoroughly cleaned to remove excess of feed and faeces, with a partial water renovation from the filter cleaning. During this period the actual feed intake per tank was recorded every 15 days. The first 7 days were used for adaptation to the feed and no faeces were collected, after that, faeces accumulated in each settling column were daily collected before the morning meal during 20 days and centrifuged at 5000 rpm for 10 minutes, discarded the supernatant, and stored at -20ºC until analysis.

2.3. Chemical analysis

Chemical analysis of ingredients, diets and faeces were conducted as follows: dry matter, by drying the samples at 105°C until constant weight; ash, by incineration in a muffle furnace at 450°C for 16 hours; protein (N x 6.25) by the Kjeldahl method following acid digestion, using Kjeltec digestion and distillation units (Tecator Systems, Höganäs, Sweden; models 1015 and 1026, respectively); lipids in ingredients and diets by extraction with petroleum ether using a Soxtec system (Tecator Systems, Höganäs, Sweden; extraction unit model 1043 and service unit model 1046), and in faeces by a gravimetric method (Folch *et al*., 1957) due to the limited amount of faeces sample; gross energy, by direct combustion of samples in an adiabatic bomb calorimeter (PARR Instruments, Moline, IL, USA; PARR model 1261); chromic oxide by acid digestion according to Furukawa and Tsukahara (1966).

2.4 Tissue sampling

A pooled sample of 10 fish at the beginning of the trial was stored at -20ºC for future whole body analysis. On day 62 of the experiment the total number of fish were individually body weighed from each tank to know the final weight per treatment. At the end of the growth trial 3 fish per tank were randomly sampled from each group 4 hours after the morning meal. Individual blood samples were withdrawn from caudal vessels using heparinized syringes. Plasma samples were obtained by centrifugation at 3000 rpm for 15 min using a high-speed microcentrifugation and kept at -70ºC for future analysis. Viscera with liver were dissected out from that three fish in each replicate tank, weighted and then, only liver was weighted to get viscerosomatic index (VSI) and hepatosomatic index (HSI) respectively. Another 3 fish from each tank were sampled for whole wet weight and length and stored at -20ºC for future composition analysis.

2.5 Evaluation of growth performance parameters

At the end of the trial, following variables per tank were evaluated:

Weight gain (% IBW) = (final weight − initial weight) × 100 / initial weight.

Weight gain (g kg ABW^{-1} day^{-1}) (WG) = ((FBW-IBW) x 1000) / (ABW x 62 days).

Specific growth rate (SGR %, day^{-1}) = {(Ln (final weight) − Ln (initial weight)) / duration (62 days)}×100.

Survival (%) = 100 × (final number of fish/ initial number of fish).

Feed intake (g kg ABW^{-1} day^{-1}) (FI) = ((feed intake (g dry matter / fish) x 1000) / (ABW x 62 days)

Daily growth index (DGI) = 100 x [$(\text{final weight})^{1/3}$ – $(\text{initial weight})^{1/3}$] x ($day^{-1}$)

Feed conversion ratio (FCR) = live weight gain (g) / dry feed intake (g).

Food efficiency (FE) = Weight gain (g) / Total intake (g)

Protein efficiency ratio (PER) = live weight gain (g) / dry protein intake (g).

2.6 Evaluation of Apparent digestibility coefficients (ADCs)

Apparent digestibility coefficients (ADCs) of protein, lipids, dry matter, and energy of the diets were determinate by the following formulas:

ADC Protein (%) = 100 x 100 [(Cr_2O_3 in diet/ Cr_2O_3 in faeces) x (Crude protein in faeces/Crude protein in diet)].

ADC Lipids (%) = 100 x 100 [(Cr_2O_3 in diet/ Cr_2O_3 in faces) x (Lipids in faeces/Lipids in diet)].

ADC Energy (%) = 100 x 100 [(Cr_2O_3 in diet/ Cr_2O_3 in faeces) x (Energy in faeces/Energy in diet)].

ADC DM (%) = 100 x (1- Cr_2O_3 in diet/ Cr_2O_3 in faeces).

2.7. Statistical analysis

Data of growth and digestibility were checked for normal distribution and homogeneity of variances. Means were then compared by one-way ANOVA followed by Tukey test at a significance level of 5%. All analyses were made using Statgraphics Centurion XVI (Statpoint technologies Inc.).

3. RESULTS

3.1. Evaluation of growth performance parameters

After 62 days the results on growth and feed utilization of European sea bass fed with the experimental diets are presented in Table 4.

During experiment no mortality of the fish was recorded. Growth performance results of fish in all the dietary treatments in the present experimental condition were satisfactory due to all fish accepted the experimental diets which were not strongly influenced by dietary formulation. The different levels of replacing of fish meal to insect meal in the three experimental diets did not affect final wt, WG, FI, FCR, FE, SGR and DGI in European sea bass (P>0.05). Just PER obtained in the control diet (HM0) showed higher level than the experimental diet with 19.5% of insect meal inclusion (HM19.5).

Table 4. Growth performance, feed efficiency and survival of European sea bass feed experimental diets with increasing levels of insect meal.

	HM0	**HM6.5**	**HM13**	**HM19.5**	**SEM***
Initial wt[1] (g)	50	50	50	50	0.13
Final wt[2] (g)	123	131	132	127	2.47
Survival (%)	100	100	100	100	
WG[3] (g)	733	816.	825	778	24.86
WG (g/kg/day)	13	14	14	14	0.24
WG (% IBW)	146	163	165	155	5.03
FI[4] (gDM/kg/day)	22.49	23.75	23.84	22.88	0.44
FCR[5]	1.65	1.64	1.64	1.62	0.02
FE[6]	0.61	0.61	0.61	0.62	0.008
SGR[7](% / day).	1.46	1.56	1.57	1.51	0.04
DGI[8]	2.09	2.26	2.28	2.18	0.05
PER[9]	1.38 **a**	1.34 **ab**	1.29 **ab**	1.27 **b**	0,01

[1]In wt: initial weight.
[2] Fn wt: final weight.
[3] WG: percent weight gain.
[4] FI: Feed Intake
[5] FCR: Feed conversion ratio
[6] FE: Feed efficiency ratio
[7] SGR: Specific growth rate
[8] DGI : Daily growth index
[9] PER: Protein efficiency ratio
* Standard error of the mean.
Values are means of triplicate groups. Within a row, means with differentletters are significantly different (*P*>0.05).

3.2 Digestibility of the diets and raw material

The apparent digestibility coefficients (ADC's) of the four diet groups were high, with ADC's of protein around 91-93%, lipids 89-92%, energy 78-83% and dry matter 66-74% (Table 5). No significant differences (P<0.05) were found between the four groups in any of the ADC's results.

Table 5. Apparent digestibility coefficients (ADC) of the four experimental diets.

Diet	HM0	HM 6.5	HM 13	HM 19.5	SEM*
ADC $_{DM}$	66.16	74.48	71.35	69.84	3.64
ADC $_{Protein}$	91.29	93.44	92.33	91.58	1.11
ADC $_{Energy}$	78.28	83.69	81.93	81.39	2.64
ADC $_{Lipids}$	89.51	91.46	92.64	92.30	1.82

* Standard error of the mean.
Data shown as mean ± SD (n=3). Figures in the same row with different letters indicate a significant difference (P < 0,05).

4. DISCUSION

The results obtained from the present study in growth parameters showed that the insect meal can be included successfully up to 19.5% without negative effects on growth and fish performance. Like in our study, Zhang *et al*., 2014 shown that in yellow catfish, 25% replacement of fishmeal by black soldier fly larva powder produced no significant difference in the growth index and immunity index when compared with those in control group without insect meal.

The results that we obtain could be explained for the addition of Taurine in the four experimental diets. Taurine is an abundant non-proteic amino acid involved in numerous physiological processes (Divakaran, 2006), also is recognized as a potent antioxidant, having an oxygen free radical scavenger effect, leading to a reduction of lipid peroxidation, reduction of membrane permeability, and reduction of membrane permeability, and reduction of intracelular oxidation and so protecting tissue form oxidative injury (Hagar, 2004; Parvez *et al*., 2008; Yu and Kim, 2009; Zeng *et al*., 2010). The taurine appear to have beneficial proprieties on the performance and well-being of carnivorous fish (El-Sayed, 2013).

In fish, under normal nutritional conditions, taurine is synthesized from methionine and cysteine metabolism, but the limited capacity to synthesize may impose the requirements for methionine and cysteine, such as during early life stage or when the dietary intake of this AA is insufficient (Buñuelos-Vargas *et al*., 2014). Apart from insect meal, the experimental diets of this trial have some partial plant feedstuffs, and some plant feedstuffs have negligible taurine levels, so fish meal replacement by these ingredients may compromise dietary levels (El-Sayed, 2013), but little information has been reported about the role of taurine on the metabolic pathway and antioxidant status of carnivorous fish, particularly its effects when supplemented in plant protein based diets (Buñuelos-Vargas *et al*., 2014). The increasing inclusion of insect meal in the experimental diets maybe could compromise the optimum levels of taurine, therefore we decided to supplement all diets with extra taurine. So in fact, this taurine extra supplementation may explain that no statistical differences were found for this growth trial between any experimental groups. Another experiment of Salze *et al*. (2010) also indicated that supplementation of methionine and lysine in addition to taurine is imperative for the complete replacement of fish meal with alternative protein sources from the diets of cobia. In this study amino acids were not added to the experimental diets so that maybe explain the significant results between the control diet (HM0) and experimental diet with an inclusion of 19.5% of insect meal (HM19.5), the decreasing levels of fish meal became deficient diets in certain amino acids such as methionine which compromise the correct use of the remaining amino acids, for that reason the lowest values of PER appeared in the experimental diet HM19.5.

Also these results shown that the feed intake between groups was the same, so no negative effects (less palatability or imbalance of amino acid) of this fish meal substitution appear with the inclusion of insect and plant proteins. However, the amino acid and palatability issue could also be overcome with amino acid supplementation or appropriate feed formulation (Kader *et al*., 2010) or also by adding natural attractants. In this case all the diets, including the highest inclusion of insect meal (19.5%) instead of fish meal, were found effective for European sea bass. The results differ when vegetable proteins are used, in the studies carry out by Kaushik *et al*. (1995) in rainbow trout (*Oncorhynchus mykiss*) and Salze *et al*. (2001) in cobia (*Rachycentron canadum*), with substitution of fish meal by soybean shown a lower feed intake with higher inclusion levels of soybean protein in diets, something that also had detrimental effects on the performances of fish. Another factor that could affect feed intake is the presence of chitin in black soldier meal (Kroeckel *et al*., 2012). In this case, no evidence of that factor had been reported but there are several study that reported a reduce of growth and feed intake, for example in rainbow trout with levels of 10% chitin in diets (Lindsay *et al*., 1984). The palatability is an important factor to measure the feed intake on fish, in this case the inclusion of insect meals without any difference in the feed intake between groups showed no effect on the acceptability of the diet with less fish meal (HM6.5, HM13, HM19.5) but sometimes the fish meal substitution reduces the palatability so is important in that cases to correct that. Kader *et al*. (2010) investigate the effect of supplementation of small amount (10%) of crude ingredients such as fish soluble (FS), squid meal (SM) and krill meal (KM) in high soy protein concentrate based diets was effective to achieve similar performance of the red sea bream as with the control diets group diet based with fishmeal. Supplementation of FS, SM and KM acted as natural feeding attractants as well as being effective in balancing amino acids and masking the unpalatable substances in diet with soy protein concentrate (Abdul Kader *et al*., 2012).

In other study from St-Hilaire *et al* (2007a) that they evaluated the use of black soldier fly in feed for rainbow trout (*Oncorhynchus mykiss*), the weight gain of fish was less for fish fed with 50% of insect meal substitution, but any significant different appear between the fish fed with diets of 25% of insect meal and the control diet without insect meal in weight gain and feed conversion ratio, the present study showed the same results for those parameters.

Lock *et al*. in 2014, obtained an increased in feed conversion efficiency in diets with black soldier meal for Atlantic salmon (*Salmo salar*), and also any differences between any dietary groups (25%, 50% and 100%) and sensory testing of fillets were found. The method of preparation of insect meal might impact on the results.

In juveniles of turbot (*Psetta maxima L.*), a marine carnivorous flatfish, Kroeckel *et al*. (2012) discovered that growth performance decreased, generally, as the percentage of insect meal in test diets increased, showed that higher specific growth rate and final body weight with the fish fed with the control diet, without insect meal, than those fish fed with any of the experimental diets containing insect meal. In terms of feed conversion, significantly higher values were obtained in the fish groups fed with higher percentage of insect meal (49%, 64% and 76%), being a negative result for the use of this alternative meal. Also like in our study the protein efficiency ratio (PER) were affected by dietary treatment and decreased significantly according increasing levels of insect meals. The authors of that study concluded that juveniles turbot could have some troubles in digesting chitin, and only a limited percentage of insect meal from black soldier fly can replace the fish meal in commercial diet for turbot.

Carnivorous fish can potentially adapt to a plant-based diet after a few generations of artificial selection (Le Boucher *et al*., 2011, 2012) so is important to continue with research like this one in the feeding area preference of fish to study the fish are able to adapt after several generation of exposure of this insect meal diets against the commercial fish meal diets. In the specie used, European sea bass, there are some studies that suggest that almost total replacement of fish meal by vegetable ingredients in diet does not affect fish growth or feed utilisation (Kaushik *et al*., 2004). On the other hand, Gomez-Requeni *et al*. (2004) found that a total substitution of fish meal reduced growth performances by about 30%.

Several experiments have shown that black soldier fly larvae inclusion in diets could partially or fully substitute for fishmeal in fish diets. However, additional trials as well as economic analyses are necessary because reduced performance has been observed in some cases, and the type of rearing substrate and the processing method affect the utilization of the larvae by fish. (Makkar *et al*., 2014).

Digestibility is the main factor in evaluating the suitability of feed ingredients for a target fish species. Usually does not vary significantly with the amount of ingested food, the number of meals (Choubert *et al*., 1984), environmental factors such as temperature and salinity, or biotic factors, such as age (Cho and Kaushik, 1990). But according with species, extrusion process of feedstuffs and factors intrinsic to the feed and the ingredients that compose it, the digestibility coefficients could vary.

Regarding to our results, in the present study we observed relatively high ADC values for lipids (89- 92%) and proteins (91-93%), suggesting a potential for these insect proteins as partial replacement for fish meal in European sea bass diets. On the other hand, lower and less homogeneous results for ADC of dry matter (66-74%) and energy (78-83%) were reported between diet groups , but not statistical differences appear.

Chemical composition of insect meal is very variable and highly dependent on processing technology and the nutritional composition of insect diet (Makkar *et al*., 2014), so this will be affect to the digestibility coefficient parameters in fish. The nutritional value and nutrient content of insect meal may be greatly affected by quality of the species of insect. Accordingly to the insect specie used in this case, black soldier fly (*Hermetia illucens*), in a trial done by Kroeckel *et al*. (2012) in juveniles of turbot (*Psetta máxima),* the calculated ADC´s for Hermetia meal were lower than this trial for all the parameters: 45.2% for organic matter, 63.1% for crude protein, 78.0% for crude lipid, and 54.5% for gross energy.

Lipids, particularly fatty acids, are the preferred source of digestible energy for fish (Tocher 2003; Lim *et al*., 2011). Insect meal used in this study had a lipid content of 5.47 % which was lower than the content in fish meal (11.3%). However, the ADC's of lipids did not showed any significantly difference between the control diet with 100% of fish meal with the rest diet with increasing levels of substitution, so all lipid sources were effectively digested (>89 %).

The results of lipids ADC were similar to the results obtained by Kroeckel *et al*. (2012) for turbot juveniles. In that study the results of control diet comparing to our control diet was higher, 98.7% due to 89.51% in the present study. But the results for 33% of insect meal comparing to ours with 19.5% of insect meal replacement were in both cases around 92 % of ADC for lipids.

The digestibility of lipids can be related with functional changes in the intestinal level induced lipids, as it known that the inclusion of vegetables oils in the diet of carnivorous fish can adversely affect the process of digestion and absorption, this can immediately notice that the inclusion up to 22.5% non-affected the digestive process (Santigosa *et al*., 2011). Also a modifications of enterocyte membranes were also described in fish fed with vegetable oils (Sitjà-Bobadilla *et al*., 2005) and this can compromise the intestinal function, thus reducing lipid digestibility (Geurden *et al*., 2009). In plant protein-based diets reduced lipid digestibility was ascribed to the soybean meal (Øverland *et al*., 2009), maybe related to a result of physiological changes in secretion of bile acid (Yamamoto *et al*., 2007), caused by the alcohol-soluble substance of soybean meal (Yamamoto *et al*., 2008).

In this study all diets were supplemented with taurine so that may explain that there was no statistical difference between the four different groups in de lipids ADC as taurine has been reported to increase the nutrient absorption, particularly the lipid fraction, through the strict dependence on taurine for bile acid conjugation and through the normalization of the intestinal morphology (Buñuelos-Vargas *et al*., 2014).

In the present study, we observed relatively high ADC values of protein for all experimental diets, something that suggested the high potential for insect meal as partial replacement for fish meal in European sea bass diets, in concordance with the reported ADC's of crude protein for fish meal in this specie, as around 90% (da Silva and Oliva-Teles, 1998).

In comparison to other studies done with alternative animal protein sources Davies *et al*. (2009) in turbot, very low result were reported for lipid and energy digestibility for poultry meat meal (ADC lipid: 54.6% and ADC energy: 45.2%), spray-dried haemoglobin meal (ADC lipid: 34.6% and ADC energy: 35.6%) and hydrolysed feather meal (ADC lipid: 37.1% and ADC energy: 21.7%) comparing to the results obtained in the present study (ADC lipid:89-92% and ADC energy:88-90%). That low results were a result of the short digestive tract and low metabolic rate of the specie selected.

In other hand, an insect experiment done by Ogunji *et al*. (2009) for determinate the ADC's of the use of horsefly maggot meal in diets for carp (*Cyprinus carpio*) was really successful with high values of ADC's (ADC lipid: 96.8%, ADC protein: 84.9%, ADC energy: 74.9%) and also close to results of this study of European sea bass. In the same study they determinate also the ADC,s in Nile tilapia with lower results than for carp (ADC lipid: 86.1%, ADC protein: 57.7%, ADC energy: 58.1%).That results are maybe explained for the presence of chitin, it has been reported that a low lipid utilization and small lipid retention at high black soldier meal inclusion levels can be attributed to the presence of chitin and its negative influence on lipid digestibility (Kroeckel *et al*., 2012). In this study the ADC levels for lipids apparently no signs that the lipid utilization was affected by the presence of chitin in the diets.

The relative low ADC's of energy (78-83%) and dry matter (66-74%) of this trial using insect meal is most certainly related to the high fibre content, as it is known that fish, specially carnivorous species, poorly digest complex carbohydrates (Enes *et al*., 2011). Indeed, non-digestible carbohydrates, present in high quantity in a diet, may also impair digestibility of other nutrients by reducing gut-retention time of feed and time available for nutrients absorption (Stone, 2003; Fountoulaki *et al*., 2005; Enes *et al*., 2011).

The results of ADC for dry matter were the lowest comparing to the rest of ADC's, 66-74%, but no significant differences between groups were found. The control diet and the other three experimental diets contain Soybean meal, wheat meal, wheat gluten and corn gluten so may be the low results are related to the presence in the vegetable feedstuff of crude fibre and non-starch polysaccharides (NSP), that are not digested by fish (Sullivan and Reigh, 1995; Lupatsch *et al*., 1997; Krogdahl *et al*., 2003).

Nevertheless, digestibility may be affected by other several factors including the fish species, fish size and water temperature (NRC, 2014), in the present study this factors did not affect the coefficient of digestibility. Further studies should be performed in order to confirm more in detail it is possible to extrapolate levels of insect meal to a good feed digestibility.

5. CONCLUSIONS

- In conclusion, juveniles of European sea bass accepted diets containing an inclusion until 19.5% of insect meal, from the specie *Hermetia illucens,* without effect on growth parameters and nutrient digestibility of the diets.

- In general, from the promising results of this study, we can confirm the possibility to use the insect meal as a substitute of fish meal in sea bass diets, helping the search for a sustainable alternative protein sources.

- So an inclusion until 19.5% (corresponding to 22.5% of the total dietary protein) in commercial diets for European sea bass is possible with the same results as using a diet for European sea bass based on fish meal, less sustainable, as the main protein source.

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6. REFERENCES

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

Barnabé, G. 1990. Rearing bass and gilthead bream. In: Barnabé, G. (ed) Aquaculture, Vol 2. Ellis Horwood, NY.

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.

Buñuelos-Vargas, I.; López, L.M.; Pérez-Jiménez, A.; Peres, H., 2014. Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on hepatic intermediary metabolism and antioxidant status of totoaba juveniles (*Totoaba macdonaldi*). Comparative Biochemistry and Physiology, Part B 170, 18-25.

Cho, C.Y. and Kaushik, S.J., 1990. Nutritional energetics in fish: energy and protein utilisation in rainbow trout (*Salmo gairdnerii*). World Review Nutrition Diets, 61, 132–172.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25-41.

Choubert, G., Blanc, J.M., Luquet, P., 1984. Influence de la modification de la fréquence de distribution des repas sur la digestibilité du régime alimentaire chez la truite arc-en-ciel. Annimal Zootechonology. 33, 255-262.

Collins, S.A., 2014. Antinutritional Factors in Modeling Plant-Based Rainbow Trout. Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada, pp. 215

da Silva, J.G. and Oliva-Teles, A., 1998. Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. Aquatic Living Resources, 11, 187–191.

Davies, S.J., Gouveia, A., Laporte, J., Woodgate, S.L., Nates, S., 2009. Nutrient digestibility profile of premium (category III grade) animal protein by-products for températe marine fish species (European sea bass, gilthead sea bream and turbot). Aquaculture Research 40, 1759–1769.

DeFoliart, G. (1992). Insects as human food. Crop Protection, 11, 395–399

Diclaro II, J.W., Kaufman, P.E., 2009. Black soldier fly Hermetia illucens Linnaeus (Insecta: Diptera: Stratiomyidae). In: EENY-461, Entomology and NematologyDepartment, Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences, University of Florida.

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.

Diener, S., Zurbrügg, C., Roa Gutiérrez, F., Nguyen Dang Hong, M.A., Koottatep, T., Tockner, K., 2011. Black soldier fly larvae for organic wastetreatment–prospects and constraints. In: WasteSafe 2011 – 2nd Int. Conf. on Solid Waste Management in the Developing Countries, 13–15 February,Khulna, Bangladesh, pp. 52–59.

Divakaran, S., 2006. Taurine: an amino acid rich in fish meal. In: Suárez, L.E.C.,Marie, D.R., Salazar, M.T., López, M.G.N., Cavazos, D.A.V., Cruz, A.C.P., García Ortega, Armando (Eds.), Avances en Nutrición Acuícola. VIII Simposium Internacional de Nutrición Acuícola. 15–17 Noviembre. Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México (ISBN 970-694-333-5).

El-Sayed, A.F.M., 2013. Is dietary taurine supplementation beneficial for farmed fish and shrimp? A comprehensive review. Rev. Aquac. 5, 1–15.

Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A., 2011. Dietary carbohydrate utilization by European sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.) juveniles. Rev. Fish. Sci. 19, 201-215.

Espe, M., Lemme, A., Petri, A., El-Mowafi, A., 2006. Can Atlantic salmon (Salmo salar) grow on diets devoid of fishmeal? Aquaculture 255, 255–262.

FAO., 2014. In: Graziano da Silva, J (Ed.). Th estate of World Fisheries and Aquaculture, Opportunities and Callenges. FAO, Rome, p.3.

Finke, M. D. (2002). Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology, 21, 269–285.

Finke, M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol. 21, 269–285

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

Fontoulaki, E., Alexis, M.N., Nengas, I., Venou, B., 2005. Effect of diet composition on nutrient digestibility and digestive enzyme levels of gilthead sea bream (*Sparus aurata* L.). Aquac Res. 36, 1243-1251.

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

Froese, R. and D. Pauly. Editors. 2015. FishBase.

Gasco, L., Gai, F., Piccolo, G., Rotolo, L., Lussiana, C., Molla, P. and Chatzifotis, S. 2014b. Substitution of fish meal by *Tenebrio molitor* meal in the diet of *Dicentrarchus labrax* juveniles. In: Insects to Feed The World, The Netherlands, 14–17 May 2014. p. 80.

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

Geurden, I., Jutfelt, F., Olsen, R.E., Sundell, K.S., 2009. A vegetable oil feeding history affects digestibility and intestinal fatty acid uptake in juvenile rainbow trout *Oncorhynchus mykiss*. Comp. Biochem. Physiol. A-Mol. Integr. Physiol. 152, 552-559.

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.

Gómez-Requeni P., Mingarro, M., Calduch-Giner, J.A., Médale, F., Martin, S.A.M, Houlihan, D.F., Kaushik, S.J. & Pérez-Sánchez, J. (2004) Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). *Aquaculture*, 232, 493-510. Gouveia, A. & Davies, S.J. (2000) Inclusion of an extrude.

Hagar, H.H., 2004. The protective effect of taurine against cyclosporine A-induced oxidative stress and hepatotoxicity in rats. Toxicol. Lett. 151, 335–343

Hardouin, J., Mahoux, G., 2003. Zootechnie d'insectes – Elevage et utilisation au bénéfice de l'homme et de certains animaux. In: Bureau pour l'Echange etla Distribution de l'Information sur le Mini-élevage (BEDIM), 164 p.

Hardy, R.W., 2002. Rainbow trout, Onchorhynchus mykiss. In: Webster, C.D., Lim, C.E. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. Harworth Press, Binghampton, New York, USA, pp. 184–202

Hasan, M.R., 2001. Nutrition and feeding for sustainable aquaculture development in the third millennium. In: Subasinghe, R.P., Bueno, P., Phillips, M.J., Hough, C., McGladdery, S.E., Arthur, J.R. (Eds.), Aquaculture in the Third Millennium. NACA, Bangkok/FAO, Rome/Bangkok, Thailand, pp. 193–219.

Howe, E.R., Simenstad, C.A., Toft, J.D., Cordell, J.R., Bollens, S.M., 2014. Macroinvertebrate prey availability and fish diet selectivity in relation to environmental variables in natural and restoring north San Francisco bay tidal marsh channels. San Franc. Estuary Waters. Sci. 12, 1–46.

Jabir, M.D.A.R., Razak, S.A., Vikineswary, S., 2012a. Nutritive potential and utilization of super worm (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., 1981. Formulation, processing and water stability of two new pellet fish feeds. Aquaculture 23, 355–359.

Jensen, M.K., S.S. Madsen and K. Kristiansen. 1998. Osmoregulation and salinity effects on the expression and activity of Na+, K+-ATPase in the gills of European sea bass, Dicentrarchus labrax (L.) Journal of experimental zoology 282: p.290-300.

Kader, M.A., Bulbul, M., Koshio, S., Ishikawa, M., Yokoyama, S., Nguyen, B.T., Komilus, C.F., 2012. Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractans supplementation in diets for red sea bream, *Pagrus major*. Aquaculture 350-353, 109-116.

Kader, M.A., Koshio, S., Ishikawa, M., Yokoyama, S., Bulbul, M., 2010. Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, Pagrus major. Aquaculture 308, 136–144.

Karapanagiotidis, I.T., 2014. The Re-Authorization of Non-Ruminant Processed Animal Proteins in European Aqua feeds. Aqua feeds. Fish Aquac. J. 5, 4.

Kaushik, S.J., 1998. Whole body amino acid composition of European seabass (Dicentrarchus labrax), gilthead seabream (Sparus aurata) and turbot (Psetta maxima) with an estimation of their IAA requirement profiles. Aquat Living Resour. 11, 355-358.

Kaushik, S.J., Covès, D., Dutto, G. & Blanc, D. (2004) Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture*, 230, 391–404.

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 2012, 345–352.

Krogdahl, A., Bakke-McKellep, A.M., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). Aquaculture Nutrition, 9, 361–371.

Leclercq, M., 1997. A propos de Hermetia illucens (Linnaeus, 1758) (Soldier fly) (Diptera Stratiomyidae: Hermetiinae). Bull. Annls. Soc. Belge. Ent. 133,275–282.

Lim, C., Yildirim-Aksoy, M. & Klesius, P. (2011) Lipid and fatty acid requirements of tilapias. N Am J Aquac, 73, 188–193.

Liu, Q., Tomberlin, J.K., Brady, J.A., Sanford, M.R., Yu, Z., 2008. Black Soldier Fly (Diptera: Stratiomyidae) larvae reduce Escherichia coli in dairy manure. Environ. Entomol. 37, 1525–1530

Lupatsch, I., Kissil, G.W., Sklan, D., Pfeffer, E., 1997. Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. Aquaculture Nutrition, 3, 81– 89.

Makkar, H.P.S.,Tran,G., Henze, V., Ankers, P., 2014. "State-of-the-art on use of insects as animal feed." Animal Feed Science and Technology 197: 1-33.

Merrifield, D.L., Olsen, R.E., Myklebust, R., Ringo, E., El-Shemy, H., 2011. Dietary effect of soybean (Glycine max) products on gut histology and microbiota of fish. In: El-Shemy, H. (Ed.), Soybean and Nutrition. InTech.

Messina, M., Piccolo, G., Tulli, F., Messina, C.M., Cardinaletti, G., Tibaldi, E., 2013. Lipid composition and metabolism of European sea bass (*Dicentrarchus labrax L.*) fed diets containing wheat gluten and legume meals as substitutes for fish meal. Aquaculture 375-379, 6-14.

Nakagaki, B. J., & Defoliart, G. R., 1991. Comparison of diets for mass-rearing Acheta domesticus (Orthoptera, Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. Journal of Economic Entomology, 84, 891–896

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 nondefatted silkworm pupae. Asian Fish Sci. 13, 235–242.

Nandeesha, M.C., Srikanth, G.K., Keshavanath, P., Varghese, T.J., Basavaraja, N., Das, S.K., 1990. Effects of non-defatted silkworm pupae in diets on the growth of common carp, Cyprinus carpio. Biol. Wastes 33, 17–23.

National Research Council (NRC), 2011. Nutrient Requirements of Fish and Shrimp. Washington, DC: National Academy Press.

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 AlternativeFeeds for Aquaculture, NOAA 15/11//2007–29/2/2008.

NRC, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC, USA

Ogunji, J.O., 2004. Alternative protein sources in diets for farmed tilapia. Nutr. Abs. Rev. B 74, 23–32

Oonincx, D.G.A.B., de Boer, I.J.M., 2012. Environmental impact of the production of mealworms as a protein source for humans – a life cycle assessment. PLoS ONE, 7.

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Skrede, Å., 2009. Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (Salmo salar)—effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. Aquaculture 288, 305–311.

Papatryphon, E., Soares, J.H., 2001. Optimizing the levels of feeding stimulants for use in high-fish meal and plant feedstuff-based diets for striped bass, Morone saxatilis. Aquaculture 202, 279–288.

Parvez, S., Tabassum, H., Banerjee, B.D., Raisuddin, S., 2008. Taurine prevents tamoxifeninduced mitochondrial oxidative damage in mice. BCPT 102, 382–387.

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

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

Peres, H., Oliva-Teles, A., 2006. Effect of the dietary essential to non-essential amino acido on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture 256, 395-402.

Piccolo, G., Marono, S., Gasco, L., Iannaccone, F., Bovera, F. and Nizza, A. 2014. Use of *Tenebrio molitor* larvae meal in diets for gilthead sea bream *Sparus aurata* juveniles. In: Insects to Feed The World, The Netherlands, 14–17 May 2014. p. 76.

Piferrer, F., M. Blázquez, L. Navarro, and A. González. 2005. Genetic, endocrine, and environmental components of sex determination and differentiation in the European sea bass (Dicentrarchus labrax L.). General and comparative endocrinology 142: p.102–10.

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

Rumpold, B.A.; Schlüter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. Innovative Food Science and Emerging Technologies 17, 1-11.

Santigosa, E., Garcia-Meilan, I., Valentin, J.M., Navarro, I., Perez-Sanchez, J., Gallardo, M.A., 2011. Plant oils' inclusion in high fish meal-substituted diets:

effect on digestion and nutrient absorption in gilthead sea bream (*Sparus aurata* L.). Aquac Res. 42, 962-974.

Schabel, H. G. (2010). Forest insects as food: A global review. In P. B. Durst, D. V. Johnson, R. N. Leslie, & K. Shono (Eds.), Forest insects as food: Humans bite back (pp. 37–64). Bangkok, Thailand: FAO

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.

Sitja-Bobadilla, A., Pena-Llopis, S., Gomez-Requeni, P., Medale, F., Kaushik, S., Perez-Sanchez, J., 2005. Effect of fish meal replacement by plant protein sources on non-specific defense mechanisms and oxidative stress in gilthead sea bream (Sparus aurata). Aquaculture. 249, 387-400.

St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., *et al*. (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. Journal of the World Aquaculture Society, 38, 309–313.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., Sealey, W., 2007a. Fly prepupae as a feedstuff for rainbow trout, Oncorhynchus mykiss. J. World Aquacult. Soc. 38, 59–67

Stone, D.A.J., 2003. Dietary carbohydrate utilization by fish. Rev. Fish. Sci. 11, 337-369.

Sullivan, J.A., and Reigh, R.C., 1995. Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone saxatilin* x *Morone chrysops*). Aquaculture, 138, 313–322.

Tacon, A.G.J., 1993. Feed Ingredients for Warmwater Fish: Fish Meal and Other Processed Feedstuffs. FAO Fisheries Circular No. 856 856

Tacon,A.G.J., 1994. Feed ingredient for carnivorous fish species: Alternatives to fishmeal and other fishery rescues. FAO Fisheries Circular, Nº. 856, pp. 64.

Tibaldi, E., Tulli, F., Corsin, F., 1996. Currents estimates of the essential aminoacid requirements of European sea bass (*D. labrax*) as determined by unconventional empirical methods. Workshop on Seabass and Seabream culture, Verona, Italy 16–18 ottobre 1996. EAS, Oestende, Belgium, pp. 320–323

Tiu, L.G., 2012. Enhancing sustainability of freshwater prawn production in Ohio. Ohio State Univ. South Cent. Newsl. Fall 11, 4.

Tocher, D.R. (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci., 11, 107–184.

van Huis, A., 2013. Potential of insects as food and feed in assuring food security. Annu. Rev. Entomol. 58, 563–583.

Veldkamp, T., van Duinkerken, G., van Huis, A., Lakemond, C.M.M., Ottevanger, E., Bosch, G., van Boekel, M.A.J.S., 2012. Insects as a sustainable feedingredient in pig and poultry diets – a feasibility study. In: Rapport 638 – Wageningen Livestock Research, Available at: http://www.wageningenur.nl/upload mm/2/8/0/f26765b9-98b2-49a7-ae43-5251c5b694f6 234247%5B1%5D.

Whitley, S.N., Bollens, S.M., 2014. Fish assemblages across a vegetation gradient in a restoring tidal freshwater wetland: Diets and potential for resource competition. Environ. Biol. Fishes 97, 659–674.

Wilson, R.P., 2002. Protein and amino acids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Elsevier Science, San Diego, USA, pp. 144–179.

Yamamoto, T., Goto, T., Kine, Y., Endo, Y., Kitaoka, Y., Sugita, T., Furuita, H., Iwashita, Y.,Suzuki, N., 2008. Effect of an alcohol extract from a defatted soybean meal supplemented with a casein-based semi-purified diet on the biliary bile status and intestinal conditions in rainbow trout Oncorhynchus mykiss (Walbaum). Aquac.Res. 39, 986–994.

Yamamoto, T., Suzuki, N., Furuita, H., Sugita, T., Tanaka, N., Goto, T., 2007. Supplemental effect of bile salts to soybean meal-based diet on growth and feed utilization of rainbow trout Oncorhynchus mykiss. Fish. Sci. 73, 123–131.

Yu, J., Kim, A.K., 2009. Effect of taurine on antioxidant enzyme system in B16F10 melanoma cells. Adv. Exp. Med. Biol. 643, 491–499.

Zeng, K., Xu, H., Chen, K., Zhu, J., Zhou, Y., Zhang, Q., Mantian, M., 2010. Effects of taurine on glutamate uptake and degradation in Müller cells under diabetic conditions via antioxidant mechanism. MCN 45, 192–199.