Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online



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ARTICLE INFO

Article history: Received 18 June 2013 Received in revised form 11 December 2013 Accepted 12 December 2013 Available online 22 December 2013

Keywords: Fish nutrition Alternative feed Insects meal Fish meal Amino acids Fatty acid

1. Introduction

(Ogunji et al., 2006).

ABSTRACT

Due to the expansion of aquaculture and the limited resources available from the sea, it is necessary to find substitutes for fish meal for use in aquaculture. We believe that the use of insect meals as an alternative source of animal protein may be an option. To use insects for this purpose, it is necessary to determine the nutritive characteristics of these insects. To determinate the potential of insects as a substitute for fish meal in fish food used in aquaculture, we examined 16 different species, 5 of them as different stage of development, of the orders Coleoptera (4), Diptera (7) and Orthoptera (5). The insect analysed have a higher proportion of fat and less protein than fish meal. With the exceptions of histidine, threonine and lysine, the insects present an amino acid profile similar to fish meal, with Diptera b being the most similar group to fish meal. However, the fatty acid content of insects is very different from that of fish meal which is rich in n-3, especially 14% EPA, 16% DHA, practically absent in insects. The insects have higher ratios of omega 6 and monounsaturated fat.

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in 2005 to US \$2000/metric ton in June 2010, and this trend is likely to continue (International Monetary Fund, 2010).

The present shortage of fish meal motivates researchers to seek new protein sources with nutritional values similar to fish meal, in particular those with similar contents of the essential amino acids, phospholipids, and fatty acids (docosahexaenoic acid and eicosapentaenoic acid) that promote optimum development, growth, and reproduction (Ayoola, 2010), which would allow aquaculture production to remain economically and environmentally sustainable over the long term.

From vegetable sources, sovbean meal is the best available vegetable protein source in terms of protein content and EAA profile. However, it is potentially limiting in sulphur-containing amino acids (methionine and cysteine) and contains some antinutrient substances such as trypsin inhibitor, haemagglutinin, and antivitamins (Tacon, 1993).

Regarding sources of animal origin mostly of them are forbidden by prescription of food security, which have made more urgent the search for alternatives to fish meal in aquaculture diets (Ogunji, 2004).

Edible insects are a natural renewable resource used as food by humans (Ramos-Elorduy and Conconi, 1994). Since ancient times, insects have been one alternative protein source used to compensate for the periodic or seasonal scarcity of other sources (Ramos-Elorduy, 1997). The most existing studies have focused on the insects that have played an important role in human nutrition in Africa, Asia, and Latin America. Thus, we can highlight the studies conducted in Nigeria (Akinnawo and Ketiku, 2000; Banjo et al., 2006), Mexico (Ramos-Elorduy, 1997; Ramos-Elorduy and Conconi, 1994; Ramos-Elorduy et al., 1997, 2006), Thailand (Yhoung-Aree et al., 1997) and Zaire (Kitsa, 1989).

Fish have been a key source of food for humans (Ayoola, 2010), but

the global catch of wild fish declined approximately 3% from 2004 to

2009 at a rate of 0.5% per year (FAO, 2010). Currently, aquaculture is

playing an essential role in the seafood market, meeting the demand

for fish that cannot be met with the wild catch. As a consequence, in

recent years (from 2004 to 2009), aquaculture production has grown

culture. It is generally added to animal diets to increase feed efficiency

and animal growth through better feed palatability; it also enhances

the uptake, digestion, and absorption of nutrients (Mile and Chapman,

2006). It is estimated that approximately 30% of the total fish catch is

converted to fish meal and fish oil for use in animal and fish feeds

creased from 10% in 1988 to approximately 45% in 2002. The increasing

global demand for and decreasing availability of fish meal has led to

sharp increases in the price of fish meal, and hence, the cost of aquacul-

ture production has increased as well (Ayoola, 2010). The price of

producing fish through aquaculture has risen from US \$600/metric ton

The percentage of fish meal that is used for aquaculture feeds has in-

Fishmeal is one of the major components of the feed used in aqua-

by 32%, a growth rate of approximately 5.6% per year (FAO, 2010).





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^{0044-8486/\$ -} see front matter © 2013 Elsevier B.V. All rights reserved. http://dx.doi.org/10.1016/j.aquaculture.2013.12.024

Although insects began to be evaluated as a potential foodstuff for animals 40 years ago (Calvert et al., 1969; Hale, 1973; Ichhponani and Malik, 1971; Newton et al., 1977; Phelps et al., 1975; Teotia and Miller, 1974), the incorporation of insects into fish feed has not received much attention until recently (Ogunji et al., 2006). In the last 10 years there have been several studies of feeding experiments performed *in vivo* with diets based on insect meal in *Clarias anguillaris* (Achionye-Nzeh and Ngwudo, 2003), *Clarias gariepinus* (Alegbeleye et al., 2012; Aniebo et al., 2011; Fasakin et al., 2003), *Orcorhychus mykiss* (Sealey et al., 2011; St-Hilaire et al., 2007) *Oreochromis niloticus* (de Haro et al., 2011a,b,c,d,e; Ogunji et al., 2006, 2008) and *Psetta maxima* (Kroeckel et al., 2012). In general, percentages of substitution higher than 30% decreased the growth depending on the fish and insect species.

From the nutritional point of view, depending on species and/or stage, insects are rich in protein and lipids, nevertheless the presence of chitin *a priori* indicates a negative characteristic. However, chitin also is present in crustacean, which are widely consumed by fish.

To evaluate the potential of insects, it is necessary to consider other advantages such as environmental benefits; the insects can be fed with waste generated by humans, having an important role in recycling materials in the terrestrial biosphere (Katayama et al., 2008). The great diversity of insect species (70–75% of animal species), from different ecosystems, with different diets and stages of development (larval, pupa, ninpha or imago) provokes a huge variability in body composition.

The aims of this study are: (1) to determine the nutritive valour of the most frequent rearing insects and some of the common species of Almeria (Spain), and (2) to establish the most similar to fish meal.

2. Materials and methods

2.1. Sampling

The insects and stages of its development (larval, pupa, ninpha or imago) studied were chosen according to the following criteria: easy to rearing, stage with mayor biomass and low exoskeleton. The wild species were chosen in terms of their availability in the environment.

The insects used in the study were obtained from pet shops (*captivity**), reared by the research group "Bionomy, Systematic and applied research on insects from University of Alicante & technologybased company Bioflytech (*captivity* **) or captured in the field close to our environment (*wild-rearing*). Table 1 summarises the insects studied, which belong to three orders, Diptera, Orthoptera and Coleoptera.

The nutritional contents of insects were compared with those of fish meal and soybean meal because they are the most common ingredients used in aquafeed production.

2.2. Analytical methods

2.2.1. Determination of proximate composition

The nutritional values obtained were derived from three replicate samples for each species of insecta, fish meal and soy meal. Moisture, crude protein, total lipids and ash were determined using AOAC (2005) techniques.

The insects were sacrificed by freezing (Finke et al., 1989). All samples, insects, fishmeal and soy meal were lyophilised (Cryodos, Ima-Telstar, Terrassa, Spain), and ground and freezing until to be analized. Total nitrogen (N) was determined using the Kjeldahl procedure, and crude protein was estimated as N x 6.25. Crude lipid was determined following the Soxhlet extraction of dried samples with petroleum ether. Moisture was determined after oven drying the samples at 105 °C to a constant weight. The ash was determined eliminating the organic matter at 500 °C during 12 h.

2.2.2. Determination of amino acid profile

The amino acid profile was determined after hydrolysing the sample with 6 N HCl for 22 h at 110 °C, followed by a sequence of filtering, derivatisation, and separation in a gas chromatograph. Tryptophan was not determined.

2.2.3. Analysis of fatty acids

For the FA analyses, all samples were transmethylated following the method of Lepage and Roy (1984) with the minor modifications of Venegas-Venegas et al. (2011): for each sample, 1 ml of freshly prepared transesterification reagents (methanol/acetyl chloride, 20:1, v/v) was added to 50 mg of freeze-dried insect meal in a glass tube along with 100 μ l of a solution of internal standard (heptadecanoic acid 17:0, 10 mg/ml). The tubes were shaken and then placed in a hot block (100 °C, 30 min). Next, the mixture was cooled to room temperature, and 1 ml of distilled water was added to each tube. The samples were shaken again and centrifuged (3,000 rpm, 3 min). The upper hexane phase was collected for GLC analysis.

The resulting FA methyl esters (FAMEs) were analysed in a Focus GLC (Thermo Electron, Cambridge, UK) equipped with a flame injection detector (FID) and an Omegawax 250 capillary column (30 m 9 0.25 mm i. d. 9 0.25 lm film thickness; Supelco, Bellefonte, PA, USA). The temperature programme was 1 min at 90 °C, heating to 200 °C at a rate of 10 °C/min, constant temperature at 200 °C (3 min), heating to 260 °C at a rate of 6C/min and constant temperature at 260 °C (5 min). The injector temperature was 250 °C with a split ratio of 50:1. The injection volume was 4 μ l. The detector temperature was 260 °C. Nitrogen was used as the carrier gas (1 ml/min).

Total saturated, monounsaturated, polyunsaturated, n-3 and n-6 fatty acids were calculated as the sums of saturated fatty acids (Σ SFA), monounsaturated fatty acids (Σ MUFA), polyunsaturated fatty acids (Σ PUFA), n-3 and n-6 fatty acids, respectively.

2.3. Data analysis

To better understand the applied value of the study's results, in addition to a descriptive approach to the nutritional value of the insects, the compositions of the insect meals were compared with the compositions of fish meal and soybean meal.

To determine the similarity between the compositions of fish meal, soybean meal and the different species of insects, a hierarchical cluster analysis was used.

Table 1
Order, stage of developement and origin of the species of insect analised.

Order	Scientific name	Stage	Abbreviation	Origin
Coleoptera	Phyllognathus excavatus	Adult	PeA C	Free-ranging
Coleoptera	Rhynchophorus ferrugineus	Larvae	RfL C	Free-ranging
Coleoptera	Tenebrio mollitor	Larvae	TmL C	Captivity*
Coleoptera	Zophoba morio	Larvae	ZmL C	Captivity*
Diptera	Calliphora vicina	Larvae	CvL D	Captivity*
Diptera	Chrysomya megacephala	Larvae (L3)	CmL D	Captivity**
Diptera	Chrysomya megacephala	Pupae	CmP D	Captivity**
Diptera	Eristalis tenax	Larvae (L3)	EtL D	Captivity**
Diptera	Hermetia illucens	Larvae (L5)	HiL D	Captivity**
Diptera	Hermetia illucens	Pupae	HiP D	Captivity**
Diptera	Lucilia sericata	Larvae (L3)	LsL D	Captivity**
Diptera	Lucilia sericata	Pupae	LsP D	Captivity**
Diptera	Musca domestica	Larvae (L3)	MdL D	Captivity**
Diptera	Musca domestica	Pupae	MdP D	Captivity**
Diptera	Protophormia terraenovae	Larvae (L3)	PtL D	Captivity**
Diptera	Protophormia terraenovae	Pupae	PtP D	Captivity**
Orthoptera	Acheta domestica	Adult	AdA O	Captivity*
Orthoptera	Anacridium aegyptium	Adult	AaA O	Free-ranging
Orthoptera	Gryllus assimilis	Adult	GaA O	Captivity*
Orthoptera	Heteracris litoralis	Adult	HIA O	Free-ranging
Orthoptera	Locusta migratoria	Adult	LmA O	Captivity*

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