Aquaculture 465 (2016) 37–42

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effect of three diets on the growth and fatty acid profile of the common ragworm *Hediste diversicolor* (O.F. Müller, 1776)

António Santos [a], Luana Granada [a], Teresa Baptista [a], Catarina Anjos [a], Tiago Simões [a], Carla Tecelão [a,b], Pedro Fidalgo e Costa [c], José Lino Costa [d], Ana Pombo [a,*]

[a] MARE – Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, 2520-641, Peniche, Portugal
[b] Linking Landscape, Environment, Agriculture and Food Research Unit (LEAF), Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, Lisbon, Portugal
[c] MARE - Marine and Environmental Sciences Centre, Laboratório Marítimo da Guia, Avenida Nossa Senhora do Cabo, 939, 2750-374 Cascais, Portugal
[d] MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 11 August 2016
Accepted 17 August 2016
Available online 24 August 2016

Keywords:
Aquaculture
Polychaete
Hediste diversicolor diets
Specific growth rate
Fatty acid profile

ABSTRACT

The polychaete *Hediste diversicolor* has a high physiological tolerance to extreme environmental factors, being easily farmed and reproduced in different types of conditions. Both in the field and under laboratory conditions, this worm can feed on different types of food. In order to highlight the potential of *H. diversicolor* for aquaculture, specific growth rate (SGR), daily growth rate (DGR), survival rate and fatty acid profile of juvenile worms, fed with three different diets, were assessed. The experiments were conducted using juvenile polychaete from a controlled reproduction with wild adults. *H. diversicolor* individuals were fed with two commercial diets, seabream dry feed (*Aquagold*) and semi-wet pellets for reared sole (*Moist Sole*), and with a non-processed diet consisting on mackerel's fillets (*Trachurus trachurus*). Juveniles fed with *Aquagold* had the highest final individual weight (0.89 ± 0.10 g). The SGR was higher in *H. diversicolor* fed with *Aquagold* and *Moist Sole*, (6.49 ± 0.30% d^{-1} and 6.54 ± 0.06% d^{-1}, respectively. The highest DGR was observed for juveniles fed with *Aquagold* (0.146 ± 0.02 g d^{-1}). The survival rate of ragworms under different treatments ranged from 96 to 100%. Regarding the protein content, the *Moist Sole* diet provided the highest percentage of protein in the reared worms (8.87%). Results showed that the total fat content of the diets was reflected in the fat content of the reared worms. The *Moist Sole* diet treatment had the highest fat content (2.25%) and individuals fed with seabream dry feed showed similar results (2.18%), while the lowest percentage was observed for the mackerel diet (0.85%). According to the fatty acid profile, the major fatty acids found in the juveniles fed with the three different diets were palmitic (C 16:0), with a higher value in the individuals fed with mackerel's fillets. Oleic (C 18:1 n9), eicosapentaenoic (C 20:5 n3), docosahexaenoic (C 22:6 n3) and stearic (C 18:0) acids presented high values in *H. diversicolor* fed with all the experimental diets.
Statement of Relevance: The common ragworm *Hediste diversicolor* is a potential high quality fatty acids source for reared fish and shrimp. Previous studies suggested that diet could be a relevant factor affecting the fatty acid composition of this polychaete (Luis and Passos, 1995). This study aimed to assess the effect of different diets on growth and survival of common ragworms juveniles (*H. diversicolor*), as well as the fatty acid profile and protein content in their tissues, aiming to find an appropriate diet to be used in commercial aquaculture.

1. Introduction

The common ragworm *Hediste diversicolor* is an Annelida Polychaeta, which inhabits the soft-bottoms of shallow marine and brackish waters in the temperate zone of the northern hemisphere, from North Africa to the North American Atlantic coast and throughout Europe (Breton et al., 2003; Scaps, 2002). Polychaetes play an important role in functioning ecosystems (Duport et al., 2006), once these individuals increase the flux of oxygen and nutrients over the sediment-water interface (Hedman et al., 2011). This is called bioturbation and leads to a modification of the physical, chemical and biological sediment properties. This species can grow and reproduce in different sediment's types, being able to tolerate extreme variations of temperature and salinity and to survive to drastic conditions of hypoxia. Moreover, it has relatively generalist feeding habits and a wide adaptation capacity to the food size, being able to behave as both deposit-feeder and suspension-feeder (Fidalgo e Costa et al., 2006). This high adaptability suggests that it is a suitable species for aquaculture.

* Corresponding author.
E-mail address: ana.pombo@ipleiria.pt (A. Pombo).

http://dx.doi.org/10.1016/j.aquaculture.2016.08.022

The increasing commercial importance of polychaetes as fishing bait and feed source in aquaculture and, consequently, their massive harvesting, are causing disturbance to the benthic community and the ecosystem (Fidalgo e Costa et al., 2000). For these reasons it is important the increase of polychaete production in aquaculture, to avoid the depletion of a natural resource and minimise the negative impacts in the environment (Nesto et al., 2012; Omena et al., 2012). Furthermore, *H. diversicolor* might be a suitable organism for integrated aquaculture since it can be a detritivorous feeder, producing valuable dietary compounds such as fatty acids from waste products from a primary fish aquaculture system. Bischoff et al. (2009) demonstrated that this species is able to recycle feed nutrients such as fatty acids when reared in an integrated recirculating aquaculture systems. The development and optimisation of new rearing techniques for *H. diversicolor* at industrial scale is crucial and one of the key steps for successful rearing of this species. In the last years, several studies have been conducted with polychaetes using different feeds, such as marine vascular and macroalgal plant sources (Nesto et al., 2012; Oliver et al., 1996), dried feed for ornamental fish Tetramin Mikromin (Tetra, Blacksburg, USA) (Fidalgo e Costa et al., 2000; Prevedelli and Vandini, 1998, 1999), phytoplankton (Riisgård et al., 1996), faeces of the carpet shell clam *Ruditapes decussatus* (Batista et al., 2003a), shrimp meat (Nielsen et al., 1995), decapsulated *Artemia* sp. cysts, sea bream dry feed and Lansy (diet for shrimp, INVE Aquaculture, Salt Lake City, USA) (Fidalgo e Costa et al., 2000). The best results were obtained with sea bream dry feed, shrimp meat and Tetramin.

During the last decades, lipids and fatty acids in particular have been more studied as they are of great importance for the food webs, and are in high demand for both human and animal nutrition (Bischoff et al., 2009). It is clear that marine fish are not capable of converting short-chain PUFA to LC-PUFA (long-chain polyunsaturated fatty acids) (Tocher et al., 1989; Tocher and Sargent, 1990; Sargent et al., 2002; Glencross, 2009; Tocher, 2010; Monroig et al., 2013; Tocher, 2015) and, thereby, LC-PUFA are considered to be essential dietary nutrients. Modes of feeding, gametogenesis and environmental temperature are some of the factors influencing the fatty acid composition of marine animals (Luis and Passos, 1995). The polychaete *H. diversicolor* is a suitable source of high quality lipids, since the fatty acid content of marine polychaetes seems to play an important role in stimulating gonad development and spawning in several reared species, such as common sole (*Solea vulgaris*), Senegalense sole (*Solea senegalensis*) and penaeid shrimp (*Penaeus kerathurus*) (Bischoff et al., 2009; Fidalgo e Costa et al., 2000; Meunpol et al., 2005; Luis and Passos, 1995) and in wild caught broodstock of *Penaeus monodon* (Vijayan et al., 2005).

The ragworm fatty acid content is important for reared fish, like sole, and for shrimp species as stated before and previous studies suggested that diet could be a relevant factor affecting the fatty acid composition of *H. diversicolor* (Luis and Passos, 1995). This study aimed to assess the effect of different diets on growth and survival of common ragworms (*H. diversicolor*), as well as the fatty acid profile and protein content in their tissues, aiming to find an appropriate feed to be used in commercial aquaculture.

2. Material and Methods

2.1. Broodstock conditioning, spawning and juvenile production

Adult *H. diversicolor* were collected from a natural population on Óbidos Lagoon, Portugal (39°24′52.7″N 9°13′13.2″W). Thirty two ragworms were placed in rearing system. The rearing system consisted of two 80 L tanks (0.175 m^2), filled with water and 15 cm of sand (250–500 μm). This was maintained with constant aeration, while temperature and salinity were kept at 25 ± 1 °C and 15, respectively. A partial water exchange was made two times a week, in order to preserve the water quality. Ragworms were reared for one month, until they matured, and then they were induced to spawn by thermal shock, decreasing temperature (5 ± 1 °C). They were fed with semi-wet pellets for cultured sole (*Moist Sole*, Sparos Lda, Olhão, Portugal) supplemented with *Ergosan*, an immunostimulant based on the macroalgae *Laminaria digitata* and *Ascophyllum nodosum*, with 0.65 g d^{-1} and 1 g d^{-1}, before and after reproduction, respectively.

1.1. Growth experiment with *H. diversicolor* juveniles

Juveniles of *H diversicolor* with similar size were selected from the reproduction assay. Ragworms were weighted (fresh weight 0.17 ± 0.03 g, mean ± SD) and placed into the tanks at a density of around 170 ind m^{-2}. The rearing system consisted of nine 80 L tanks (0.175 m^2) (three replicates per treatment), filled with 15 cm of sand (250–500 μm) and water and 30 polychaetes were stocked per tank. This system was maintained with constant aeration, whereas temperature and salinity were kept at 25 ± 1 °C and 15, respectively (Fidalgo e Costa et al., 2000; Nielsen et al., 1995). Mortality was recorded daily and a partial water exchange was made every week. The ammonium concentration was monitored during the experiment with multiparameter photometer HANNA HI 83203. The ammonium concentration was the highest in the tanks of ragworms fed on mackerel's fillets and *Moist Sole* diet treatments (4.0 mg L^{-1}). On the contrary, in tanks where *Aquagold* were applied, the ammonium concentration achieved was lower (2.0 mg L^{-1}).

Ragworms were fed with one of the three types of food for 60 days. The diets consisted of two commercial diets, seabream dry feed (*Aquagold*, Sorgal SA, Ovar, Portugal) and semi-wet pellets for cultured sole (*Moist Sole*, Sparos Lda, Olhão, Portugal), and a non-processed diet of mackerel's fillets (*Trachurus trachurus*). It was used a daily feeding rate of 3% total tank biomass, adjusted throughout the trial. The commercial diets, *Aquagold* (protein: 46.00%; Lipids: 18.00%) and *Moist Sole* (protein: 52.12%; Lipids: 20.03%), were developed by Sorgal SA and Sparos Lda, respectively. The biochemical composition for mackerel's fillets (*Trachurus trachurus*) (protein: 19.00%; Lipids: 2.50%) was similar to the results reported by Batista et al. (2008) (19.70%; Lipids: 2.90%). To further evaluate the nutritional value of dietary treatments used during experimental period, it was determined the fatty acid profile of the diets.

At the end of the experiment, following a gentle sieving, all individuals were weighted. The specific growth rate (SGR): % d^{-1} = 100 [ln (final wet weight) − ln (initial wet weight)] / duration and the daily growth rate (DGR): g d^{-1} = (final wet weight − initial wet weight) / duration were calculated according to Batista et al. (2003b) and Fidalgo e Costa et al. (2000). The crude protein, total lipid content and fatty acid profile were estimate with sub-samples of juveniles fed the experimental diets at the end of the on-growing period (three replicates per dietary treatment).

2.2. Protein content analysis

The Kjeldahl method (model Kjeltech 2006, Foss Tecator, Hillerod, Denmark) was used to determine the nitrogen content. The crude protein was estimated based on the equation: Protein content (%) = [(Va − Vb) × NHCl × 6.25 × 0.014] / m × 100, where Va is the titration volume of the sample, Vb is the titration volume of the blank, NHCl is the normality of HCl solution (0.1 N), 6.25 is the nitrogen conversion factor, 0.014 is the milliequivalent weight of nitrogen, and m stands for sample weight (g).

2.3. Lipid extraction and fatty acid profile

Total lipid extraction method was adapted from Bligh and Dyer (1959) following a dry matter basis. Fatty acid methyl esters were prepared according to the methods of Lepage and Roy (1986) and Masood et al. (2005). 0.015 mg of crude fat was dissolved in 5 mL acetyl chloride:methanol (1:19 v/v) and heated in a water bath at 80 °C for 1 h. Then, 1 mL ultrapure water and 2 mL n-heptane were added and