



Fatty Acid Profile of Wild and Farmed Sandworms, *Perinereis nuntia*, in the Coast of Bandar Abbas, Iran

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Received 21 September 2016
Accepted 07 March 2017

Abstract

To investigate the effect of different food items on fatty acids profile of *Perinereis nuntia* worms, Polychaete worms mentioned above were collected from the tidal shores of Bandar Abbas. In this study three treatments included nutritional Commercial post larval shrimp, green algae (*Enteromorpha* sp.) and red algae (*Gracillaria* sp) each with three replications dry was used to feed the worms. Feeding twice daily and 10% of the body weight of worm for two months was conducted. The results showed that different treatments in terms of food fatty acid profile there is a significant difference ($P < 0.05$). Most fatty acids (SFA) 35.88 ± 0.01 , (MUFA) 43.82 ± 0.01 and (PUFA) 108.98 ± 0.01 (mg g⁻¹) of polychaete worms were fed red algae. Results suggest that *Perinereis nuntia* fed red algae have a well-balanced nutritional profile for penaeid shrimp and fish broodstock.

Keywords: *Perinereis nuntia*, fatty Acid profile, sandworms, Bandar Abbas.

Introduction

Polychaetes have recently gained commercial importance because they are used as bait for sport, professional fishing, and as a food source in aquaculture in the blast frozen form, or as a constituent of formulated feeds. They are especially important as a maturation diet for shrimp broodstock (Gambi *et al.*, 1994; Olive, 1994; Poltana *et al.*, 2007). Commercial harvesting of polychaetes causes disturbance in the benthic community and the ecosystem (Gambi *et al.*, 1994). Most of the polychaetes used in hatcheries are wild-caught. An intense harvesting from the wild may seriously deplete the natural sources of this worm. Polychaetes feed on detritus on the shore and mangrove areas and therefore play an important role in maintaining a clean and natural environment. In addition, polychaetes lie almost at the bottom of the food chain for aquatic animals and have served this ecologically sensitive role for millions of years (Poltana *et al.*, 2007). Commercial rearing of polychaetes offers an ecologically sound solution to reduce potential environmental problems arising from the harvesting of specimens from the wild (Fidalgo *et al.*, 2000).

The nutritional value of polychaetes has been specifically attributed to their high content of PUFAs (Olive, 1999; Shucksmith *et al.*, 2006), which is an

important means to transfer essential fatty acids to fish and crustaceans in aquaculture (Bischoff *et al.*, 2009; Palmer *et al.*, 2014). For this reason, shrimp hatcheries still use natural organisms as live feed for broodstock, including sandworms, since they contain high levels of unsaturated fatty acids, other phospholipids, hormones like prostaglandin, and protein. In particular, n-3 and n-6 PUFAs are believed to be essential for the development of the shrimp reproductive system (Lytle *et al.*, 1990; Marsden *et al.*, 1997; Naessens *et al.*, 1997). Indeed, sandworms (polychaetes) are used as live feed for shrimp broodstocks to obtain better maturation and oocyte and sperm production (Wouters *et al.*, 2001). There have been many recent studies documenting the nutritional benefits of polychaetes for aquaculture species. These benefits include satisfactory food intake and reproductive performance in broodstock sole (Cardinaletti *et al.*, 2009), provision of PUFAs like AA, EPA, DHA, and the high n3:n6 ratio that help in fertilization, hatch rates, and spawning frequency in penaeid shrimp broodstock (Huang *et al.*, 2008).

The purpose of this study is to determine the fatty acid content of farmed sandworms (*Perinereis nuntia*) fed on different diets and to compare this with locally harvested wild *P. nuntia* sandworms in order to evaluate their nutritive value as a live feed in

aquaculture.

Materials and Methods

Around 225 sandworms (average weight of 0.626 g/worm) were fed with three different types of food for 60 days. There were three replicates, with 25 worms per replicate, for each diet. The diets were: commercial post larval food, green macro algae (*Enteromorpha* sp.), and red macro algae (*Gracilaria* sp.). Then, the farmed sandworms were starved for two days to clear the digestive tract before putting them on ice and then frozen at -70°C until used following the procedure of Techapremprecha *et al.* (2011). Live wild sandworms were obtained from the Bandar Abbas beach, Hormozgan province, Iran, in August 2015 and starved for two days before putting them on ice and freezing them at -70°C . These experiments were carried out under laboratory conditions in plastic baskets ($80 \times 40 \times 30$ cm) filled with fine sand up to a depth of 15 cm with a similar height of water above this. Natural sand ($250\text{--}500\mu\text{m}$) was dried in a heater at 90°C for 24 hours to kill any organisms present. The baskets were placed in tanks with static water. The baskets were aerated and the water changed daily to maintain good quality. The baskets were examined daily, the dead animals were removed, and the number recorded. The photoperiod was adjusted to 16L:8D with an average light intensity of 175 Lux (Fidalgo *et al.*, 2000). Temperature and salinity were maintained at $28\pm 2^{\circ}\text{C}$ and 41‰, respectively. At the end of the experiment, all the worms were weighed (fresh weight) and the growth was expressed thus: the specific growth rate (μd^{-1}), according to Jorgensen's (1990) formula:

$$\mu = \ln(W_t - W_0) t^{-1}$$

where, W_0 and W_t are the average biomass of the polychaetes on day zero and day t , respectively, divided by the number of days (60). This measure of growth has been used previously in case of *N. diversicolor* by Jorgensen (1990), Riisgard (1991), Vedel and Riisgard (1993), and Nielsen *et al.* (1995).

Fatty acid compositions were determined by capillary gas chromatography (GC) following the Fidalgo *et al.* (2000). The lipid extracts were saponified for 40 minutes under nitrogen at 100°C with 0.5M KOH in methanol. The resultant fatty acids were recovered and their methyl esters prepared by reaction with a 14% $\text{BF}_3/\text{CH}_3\text{OH}$ solution for 8 minutes at 100°C under nitrogen (Metcalf and Schmitz, 1961). The fatty acid methyl esters, after solvent evaporation, were recovered in 2 ml of iso-octane. They were analysed by GC (Varian 3300) using a split 12:1 and separated on a 30 m WCOT SP1000 fused silica 0.32 mm i.d. capillary column, operated for 7 minutes at 180°C then programmed at $^{\circ}\text{C} \text{min}^{-1}$ to 200°C with 1 ml min^{-1} helium carrier gas. The injector port and Flame Ionization Detector (FID)

were kept at 250°C . Column performance was monitored by routine injection with a secondary standard of fatty acid methyl esters prepared from cod liver oil.

Statistical analysis results are expressed as the mean \pm SE of sample analysis. Analysis of variance (one-way ANOVA) was used to determine statistical differences between groups. The Duncan test was performed for multiple comparisons. All references to significant differences are at a level of 5% ($P < 0.05$)

Results

The fatty acid composition of wild worms and the farmed worms is given in Table 1 where they are grouped as SFA, MUFA, and PUFA. The majority of the fatty acids in *Perinereis nuntia* in all tests were C20:0, C20:1(n-9), C20:5(n-3), and C22:6(n-3). Lesser amounts were C14:0, C16:1, C18:2(n-6), and C18:3(n-3). The analysis of mean SFA, MUFA, and PUFA components revealed a slight difference in four groups ($P < 0.05$). The contents of the mean SFA, MUFA, and PUFA were higher in the worms that were fed red algae. The major MUFA, C20:1(n-9) (Gondoic acid) had a significantly higher presence in the groups. In all the groups of worms, C22:6(n-3) (DHA) was clearly the major PUFA. However, the PUFA composition within four groups of sandworms was spread with three PUFA dominating (C22:6(n-3), C20:5(n-3), and C20:4(n-6)). The AA, DHA, and EPA levels were not different between the wild worms and the group of sandworms that were fed red algae ($P > 0.05$).

Discussion

The intensive use of marine polychaetes as fishing bait, and for enhancing reproduction in shrimp and fish broodstock in numerous countries across the world (Palmer *et al.*, 2014), represents a stress on the biodiversity of the benthic communities, particularly the polychaete community. These conditions promote the mass culture of polychaetes for commercial use (Palmer, 2010; Limsuwatthanathamrong *et al.*, 2012) in aquaculture, where they form 5%–33% of shrimp diets (Meunpol *et al.*, 2005; Coman *et al.*, 2007) relative to their advantages and preferences for use over other broodstock feeds (Palmer *et al.*, 2014). The preference of polychaetes as feed for crustaceans is attributed to their contents of a suitable balance of nutrients and several other factors promoting crustacean reproduction and larval quality (Palmer *et al.*, 2014). As in other previous studies by several authors, the present work found high concentrations of PUFA (particularly Omega-3 and Omega-6), which have important roles to play in membrane structures, metabolic processes, and are precursors to biosynthetic pathways involved in the *in vivo* supply of sex steroids (Izquierdo *et al.*, 2001; Nguyen *et al.*, 2012; Palmer *et al.*, 2014). For example, the ARA

Table 1. Mean (\pm SE; n=6) fatty acid contents* (mg g⁻¹) of wild worms of *Perinereis nuntia* and the worms fed on different diets

Fatty acid diet	Fatty acid profile			
	Commercial post larval food	Green algae	Red algae	Wild worms
C14:0	5.22 \pm .01 ^c	4.23 \pm 0.01 ^d	5.72 \pm 0.01 ^a	5.33 \pm 0.04 ^b
C16:0	6.88 \pm .01 ^a	5.88 \pm 0.06 ^d	6.83 \pm 0.01 ^b	6.41 \pm 0.01 ^c
C18:0	8.53 \pm .01 ^b	6.25 \pm 0.06 ^d	8.62 \pm 0 ^a	7.13 \pm 0.01 ^c
C20:0	12.56 \pm .01 ^b	9.57 \pm 0.01 ^d	14.72 \pm 0.01 ^a	12.20 \pm 0.03 ^c
Total SFA	33.19 \pm 0.01 ^b	25.94 \pm 0.01 ^d	35.89 \pm 0.01 ^a	31.07 \pm 0.02 ^c
C16:1	7.74 \pm 0 ^b	6.12 \pm 0.01 ^d	8.32 \pm 0.01	6.53 \pm 0.02 ^c
C18:1(n-9)	9.23 \pm 0.01 ^b	7.23 \pm 0.01 ^d	9.52 \pm 0.01 ^a	8.24 \pm 0.01 ^c
C18:1(n-7)	9.65 \pm 0.01 ^b	7.67 \pm 0.01 ^d	10.75 \pm 0.01 ^a	9.25 \pm 0.01 ^c
C20:1(n-9)	15.92 \pm 0 ^a	10.22 \pm 0 ^d	15.24 \pm 0.01 ^b	13.72 \pm 0.01 ^c
Total MUFA	42.53 \pm 0.01 ^b	31.25 \pm 0.01 ^d	43.83 \pm 0.01 ^a	37.74 \pm 0 ^c
C18:2(n-6)	10.56 \pm 0.01 ^b	8.53 \pm 0.01 ^d	11.53 \pm 0.01 ^a	10.02 \pm 0 ^c
C18:3(n-3)	11.24 \pm 0.01 ^b	9.02 \pm 0.01 ^d	12.88 \pm 0.01 ^a	10.53 \pm 0.02 ^c
C18:4(n-3)	11.85 \pm 0.01 ^b	9.52 \pm 0 ^d	13.88 \pm 0.01 ^a	11.45 \pm 0.01 ^c
C20:4(n-3)	13.23 \pm 0.01 ^c	11.53 \pm 0.01 ^d	16.53 \pm 0.01 ^a	15.25 \pm 0.01 ^b
C20:4(n-6) (AA)	14.25 \pm 0 ^b	12.92 \pm 0.01 ^c	17.22 \pm .01 ^a	17.27 \pm 0.07 ^a
C20:5n-3 (EPA)	15.18 \pm 0 ^d	15.46 \pm 0.01 ^c	18.22 \pm 0.01 ^b	18.31 \pm 0.01 ^a
C22:6n-3 (DHA)	16.74 \pm 0.01 ^c	15.81 \pm 0 ^d	18.72 \pm 0.01 ^b	18.74 \pm 0.01 ^{ab}
Total PUFA	93.06 \pm 0.01 ^c	82.78 \pm 0.01 ^d	108.98 \pm 0.01 ^a	101.57 \pm 0.02 ^b
DHA/EPA	1.1	1.02	1.02	1.02
n3:n6 ratio	2.75	2.85	2.79	2.72

* Different superscripts within rows indicate significant ($P < 0.05$) differences.

level was found to be relatively high here in *P. nuntia*. It is involved in the synthesis of prostaglandins, which have a broad set of physiological roles including the regulation of reproduction in crustaceans (Harrison, 1990; Wouters *et al.*, 2001; Meunpol *et al.*, 2010). Several polychaetes, including some from this genus (*Perinereis*) have been shown to contain these and other hormones that stimulate ovarian development in one of the more difficult penaeid species to breed, namely *P. monodon* (e.g. prostaglandin: Poltana *et al.*, 2005; progesterone, 17 α -hydroxyprogesterone: Meunpol *et al.*, 2007, 2010; methyl farnesoate: Laufer *et al.*, 1997). However, the nutritional value and PUFA profile of polychaetes as well as other diets can vary with species, season of harvest, life stage, habitat, environment, and food chain of each polychaete (Luis and Passos, 1995; Meunpol *et al.*, 2005; Chimsung, 2014). The Fatty Acid Profile of other wild polychaetes also varies, such as *N. diversicolor*, a rag worm, which had a total PUFA, AA, DHA, and EPA of 450, 31.6, 8.0, and 78.8 g kg⁻¹, respectively (Luis and Passos, 1995; Fidalgo *et al.*, 2000). The fatty acid composition of the rag worms is reflected in its diet (Fidalgo *et al.*, 2000). The abundance of fatty acids in *P. nuntia* during the present study appeared to be affected by different diets. On the other hand, the feeding habit of polychaetes may influence their fatty acid contents, particularly EPA that is common in marine diatoms and algae (Wen and Chen, 2003; Pratoomyot *et al.*, 2005, Dawczynski *et al.*, 2007; Van Ginneken *et al.*, 2011; Pereira *et al.*, 2012). A high level of EPA was also reported in *P. cultrifera*. *P. helleri*, grown in sand filters of mariculture wastewater, and fed

predominantly on microalgae also showed high levels of EPA (Palmer *et al.*, 2014). Highly unsaturated fatty acids (HUFA), especially 20:5n-3 and 22:6n-3, may be important components of live and formulated maturation diets causing their abundance in ovarian tissues (Meena *et al.*, 2013). Diets deficient in n-3 HUFA displayed a negative effect on ovarian development, fecundity, and egg quality (Wouters *et al.*, 1999). Huang *et al.* (2008) also found that in *P. monodon* broodstock fed with diets containing the highest levels of AA, EPA, DHA, and n3:n6 ratio resulted in the highest fertilization, hatch rates, and spawning frequency. In this study, the AA, DHA, and EPA contents were not significantly different between the wild worms and the group of sandworms that were fed on red algae ($P > 0.05$).

Palmitic acid was reported as the most prevalent saturate in many polychaete species followed by stearic acid (Palmer *et al.*, 2014). In the present study, stearic acid was in the second place in *P. nuntia*. It revealed that the fatty acid composition of *P. nuntia*, which was fed on red algae, was quite different from other diets except wild worms. But generally, it may be very suitable for use as feed in aquaculture. *P. nuntia* was characterized as having particularly high levels of EPA, DHA, and AA similar to the wild worms, which makes it a useful supplement for these fatty acids for a vast array of species currently cultured around the world.

Conclusions

The overall fatty acid value of these farmed sandworms fed on red algae is more appropriate as a

diet for shrimp broodstocks than the wild sandworms. However, farmed sandworms should be improved in fatty acid contents for better nutritional value. Furthermore, the use of farmed sandworms will reduce the destruction to the environment from over-harvesting of native sandworms.

Acknowledgment

This work has been conducted in the laboratory of the Persian Gulf and Oman Sea Ecology Research Center, Bandar Abbas, Iran.

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