A comparative study on the nutritional value of three Polychaete species used in Shrimp Aquaculture.

Un estudio comparativo sobre el valor nutricional de tres especies de poliquetos utilizadas en la acuicultura del camarón

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ABSTRACT

The potential use of polychaete worms are as a dietary source of protein, lipid, amino acids, and vitamins for aquaculture species and also used as alternative feeds of fish meal and fish oils. Many recent studies have been documented the nutritional benefits of polychaetes for aquaculture species, for their satisfactory food intake and reproductive performance in brood stock. Most of the polychaetes were used in hatcheries are wild caught. The commercial harvesting of polychaetes may leads to the disturbance in the benthic community and the ecosystem and also may leads to the depleting population in the ecosystem. Further the development of polychaete culture is therefore of economic importance beyond that the associated bait supply industry which is acted as the initial catalyst for this development. Hence, the present study has been made with three different polychaete species, to assess the nutritional value of the three species, one was imported (*Nereis virens*) and two locally available species (*Perinereis cultrifera* and *Glycera* sp) for commercial uses in the shrimp aquaculture industry. The samples were collected from mandapam and Palk Bay Area and these samples were oven-dried and powdered. All the samples were analyzed and calculated in dry weight per 100 grams of sample. Moisture content was calculated by loss of drying (LOD) method. Protein and amino acids were estimated by Appendix IV (British Pharmacopeia 2007), and detailed amino acid profile was assessed by HPLC. Carbohydrates were determined by gravimetry method from Appendix IV in Volume 3 Indian Pharmacopeia (2007). Total fatty acid and fatty acid profile were estimated through gas chromatography based on British Pharmacopeia (2007). Minerals were analyzed by flame photometry and atomic spectrometry (Indian Pharmacopeia, 2007; US pharmacopeia by Lawrence Evans, USP 29 NF 22 vol. 28). Vitamins were determined by assay method using liquid chromatography in US pharmacopeia (Lawrence Evans, USP 29 NF 22 vol. 28). Ash content was estimated in a tared silica crucible at a temperature of 400 to 500° C in a muffle furnace. Fibers was estimated by an enzymatic–gravimetric method followed by AOAC method 985.29 (1990). In the present study, among the three polychaete species, the maximum moisture content was recorded in *Perinereis cultrifera* (5.60 g) followed by *Glycera* sp (5.34 g) and *Nereis virens* (3.89 g) and the maximum value of protein content was recorded in *P. cultrifera* (53.62 g) followed by *Glycera* sp (50.50 g) and *N. virens* (49.04 g). The maximum total fatty acid content was recorded in *N. virens* (32.31 g) followed by *Glycera* sp (20.99 g) and *P. cultrifera* (11.99 g). The result of major fatty acids profile, showed that the Saturated Fatty Acid (SFA) content was higher when it compare to PUFA and MUFA in all three polychaetes. The maximum ash content was recorded in *Glycera* sp (20.32 g) followed by *N. virens* (11.73 g) and *P. cultrifera* (10.91 g) and the maximum carbohydrate content was recorded in *P. cultrifera* (17.53 g) followed by *N. virens* (2.67 g) and
Glycera sp (2.04 g). In this study, EAA was recorded higher content when it compared to NEAA in all the three species of polychaete. In the EAA the most dominant amino acids are lysine, arginine, and isoleucine and in the NEAA are cysteine, alanine, asparagine, and aspartic acid whereas the lesser EAA and NEAA are methionine and serine, respectively. In vitamin study, P. cultrifera, N. virens and Glycera sp showed higher content of vitamins A, B1, and E and less in vitamin C. Mineral content was dominant with chloride of all the three species of polychaete.

Key words : Polychaete worms, Perenereis species, fatty acids. amino acids, Minerals.
en comparación con NEAA en las tres especies de poliquetos. En el EAA, los aminoácidos más dominantes son lisina, arginina e isoleucina y en el NEAA son cisteína, alanina, asparagina y ácido aspártico, mientras que los EAA y NEAA menores son metionina y serina, respectivamente. En el estudio de vitaminas, P. cultrifera, N. virens y Glycera sp mostraron un mayor contenido de vitaminas A, B1 y E y menos de vitamina C. El contenido de minerales fue dominante con el cloruro de las tres especies de poliquetos.

Palabras clave: gusanos poliquetos, especies de Perenereis, ácidos grasos. aminoácidos, minerales

INTRODUCTION

Polychaete worms or marine worms are belonging to the phylum Annelida and also the dominant group of the organisms in the benthic marine environments (Stoner and Acevedo, 1990). Navedo and Masero (2007) reported that the Polychaete worms are play a major role in the marine food chains and it was found in almost all marine ecosystems. Scaps (2003) stated that the worms are regular source of food for all vertebrates, and invertebrates. Polychaetes perform many important environmental services such as sediment bioturbation (Scaps, 2002), decomposition of organic matter, breaking down of toxic ammonia-based compounds in the sediment (Kristensen et al., 1985), release of nutrients back to the environment (Scaps, 2002, Batista et al., 2003) and aeration of the sediment (Beesley et al., 2000). Shiau (1998) reported that the nutritional requirements of brood stock are less understood than those of the other penaeid shrimps production. Intensive study has been made to develop the suitable artificial diet for brood stock maturation (Coman et al., 2007; Marsden et al., 1997; Meunpol et al., 2005). Generally, in hatcheries, the fresh feed was used for brood stock growth, and these fresh feeds consist of squid, oysters, krill, artemia, and polychaetes. In the shrimps hatcheries the polychaetes worms are the most preferred food for healthy shrimp brood stocks maintenance. Soraphat Panakorn (2015) reported that the polychaetes are produced beneficial enzymes, amino acids, and hormones to stimulate the reproduction. Therefore, the live polychaetes are extensively used as feed in aquaculture industry, which is results in excellent source of polyunsaturated fatty acids (PUFAs), and hence the nereid worms are commonly called as omega worms due to their high content of omega-3 (ω-3) PUF (Lytle et al., 1990; Olive et al., 1992, Murugesan et al., 2011). The commercial value of the most exploited species of polychaetes has been documented by various authors (Klawe and Dickie, 1957; Creaser et al., 1983; Sarda Borroy, 1989; Chen, 1990). In India, the collection of polychaetes has emerged as an artisanal fishery in many coastal states and the annual consumption of these polychaetes by shrimp hatcheries is estimated to be about 6 to 20 tonnes (Vijayan et al., 2005).

The potential use of polychaete worms are as a dietary source of protein, lipid, amino acids, and vitamins for aquaculture species and also used for alternative feeds of fish meal and fish oils (Olive, 1999). Many recent studies have been documented the nutritional benefits of polychaetes for aquaculture species, for their satisfactory food intake and reproductive performance in brood stock sole (Cardinaletti et al. 2009), provision of PUFAs like AA, EPA, and DHA, and the high n3:n6 ratio that helps in fertilization, hatch rates and spawning frequency in the shrimp Penaeus sp brood stock (Huang et al. 2008). Most of the polychaetes used in hatcheries are wild caught. The commercial harvesting of polychaetes may leads to the disturbance in the benthic community and the ecosystem (Gambi et al., 1994). The continuous harvesting of polychaetes from the wild may leads to the depleting population in the ecosystem.
Further the development of polychaete culture is therefore of economic importance beyond that the associated bait supply industry which is acted as the initial catalyst for this development (Olive, 1999). Hence, the present study has been made with three different polychaete species, to assess the nutritional value of the three species, one from imported (*Nereis virens*) and two locally available species (*Perinereis cultrifera* and *Glycera* sp) for commercial uses of the shrimp aquaculture industry.

**MATERIALS AND METHODS**

This study was made with three different polychaete species for analysis: *Nereis virens* was imported from the Netherlands; *Perinereis cultrifera* and *Glycera* sp were collected from Mandapam coast and Palk Bay. The samples were collected by digging using shovel, and the polychaetes were carefully handpicked and washed gently in seawater and were starved for two days to clear the digestive tract before drying. The samples were oven-dried and powdered. All the samples were analyzed and calculated in dry weight per 100 grams of sample. Moisture is calculated by loss of drying (LOD) method by taking known initial weight of sample was accurately weighed in a tared dish and dried in an oven at 105°C for 1 hour or constant weight. It was cooled in a desiccator and weighed. LOD was calculated with reference to the amount of dried powder initially taken. Protein and amino acids were estimated by Appendix IV (British Pharmacopeia 2007), and detailed amino acid profile was assessed by HPLC. Carbohydrates were determined by gravimetry method from Appendix IV in Volume 3 Indian Pharmacopeia (2007). Total fatty acid and fatty acid profile were estimated through gas chromatography based on British Pharmacopeia (2007). Minerals were analyzed by flame photometry and atomic spectrometry (Indian Pharmacopeia, 2007; US pharmacopeia by Lawrence Evans, USP 29 NF 22 vol. 28). Vitamins were determined by assay method using liquid chromatography in US pharmacopeia (Lawrence Evans, USP 29 NF 22 vol. 28). Ash content is calculated by 1 g of sample accurately weighed which was incinerated in a tared silica crucible at a temperature of 400 to 500°C in a muffle furnace until it turns white, indicating the absence of carbon. Ash was cooled in a desiccator and weighed without delay, and the percentage was calculated on the basis of sample taken initially. Fibers was estimated by an enzymatic–gravimetric method followed by AOAC method 985.29 (1990).

**RESULT AND DISCUSSIONS**

In the present study, among the three polychaete species, the maximum moisture content was recorded in *Perinereis cultrifera* (5.60 g) followed by *Glycera* sp (5.34 g) and *Nereis virens* (3.89 g). The maximum value of protein content was recorded in *P. cultrifera* (53.62 g) followed by *Glycera* sp (50.50 g) and *N. virens* (49.04 g). This similar observation has been made in *Perinereis cultrifera* from Egyptian Mediterranean Coast (Dorgham et al., 2015), in *P. nuntia* (Techaprempreecha et al., 2011) and *N. virens* (Brown et al., 2011). In the present study the fatty acids were estimated in all the three species of polychaete. The maximum total fatty acid content was recorded in *N. virens* (32.31 g) followed by *Glycera* sp (20.99 g) and *P. cultrifera* (11.99 g) and the major fatty acids profile (Fig. 3) composition was grouped in three polychaete species as Saturated Fatty Acid (SFA), it was reported with Palmic acid (16:0), it was showed that 4.367 g, 3.030 g, and 1.067 g; Margaric acid (17:0) was showed that 2.019 g, 1.220 g, and 0.246 g; and the Stearic acid (18:0) was showed that 8.640 g, 4.580 g, and 4.055 g, in *N. virens*, *Glycera* sp, and *P. cultrifera*, respectively. Monounsaturated Fatty Acid
(MUFA) was reported with Oleic acid (18:1) and the maximum value was recorded in *N. virens* (8.030 g) followed by *Glycera* sp (3.130 g) and *P. cultrifera* (2.344 g); Polyunsaturated Fatty Acid (PUFA) was reported with Linoleic acid (18:2) and the maximum value was recorded in *Glycera* sp (5.310 g) followed by *P. cultrifera* (1.992 g) and *N. virens* (1.330 g); Alpha-linolenic acid (18:3) the maximum content was recorded in *N. virens* (6.830 g) followed by *Glycera* sp (3.210 g) and *P. cultrifera* (2.056 g); and finally, Morotic acid (18:4) the maximum content was recorded in *N. virens* (0.993 g) followed by *Glycera* sp (0.310 g) and *P. cultrifera* (0.111 g). In the present study the result of major fatty acids profile, showed that the Saturated Fatty Acid (SFA) content was higher when it compare to PUFA and MUFA in all three polychaetes and the maximum content was recorded in *N. virens* (15.026 g, 9.153 g, and 8.030 g) followed by *Glycera* sp (8.830 g, 8.830 g, and 3.130 g) and *P. cultrifera* (5.368 g, 4.159 g, and 2.344 g), respectively. This SFA was dominant with stearic acid followed by palmitic acid and this similar has been made in *P. cultrifera* (Dorgham *et al*., 2015). This similar observation has been made by various authors in *Nereis diversicolor* (Bischoff, 2009) and in *Nereis* sp (Sunil Kumar Sahu *et al*., 2017) of Palmitic acid and Oleic acid. Among the PUFA group, alpha-linolenic acid was found higher in *N. virens* and this similar observation was found in *Nereis* sp (Sunil Kumar Sahu *et al*., 2017). The PUFA (particularly Omega-3 and Omega-6) plays an important role in the membrane structures, metabolic processes, and precursors to biosynthetic pathways to involve in the in vivo supply of the sex steroids (Izquierdo *et al*., 2001; Nguyen *et al*., 2012; Palmer *et al*., 2014). Recently, polychaete fatty acids were evaluated as potential inhibitor against human glioblastoma multiforme (Reena Singh *et al*., 2013). Hence, the polychaete worms are an important vector for the transfer of essential fatty acids to fish and crustaceans in aquaculture.

![Fig. 1. Comparative analysis of proximate composition of three polychaete species (per 100 grams of each sample)](image)

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been made in *P. cultrifera* from the Egyptian Mediterranean Coast (Dorgham *et al*., 2015), in *Nereis* sp (Sunil Kumar Sahu *et al*., 2017) and in *N. virens* (Brown *et al*., 2011). Carbohydrates are also excellent binders in diet formulation (Wouters *et al*., 2001) and play an important role in glycogen accumulation in the hepatopancreas and also act as binders and transport of nutrients in the hemolymph (Harrison, 1997). The complex sugars and polysaccharides are used more effectively than simple sugars (Deshimaru and Yone, 1978). However, they can be used as source of energy with protein-sparing and lipid-sparing effects.

![Fig. 2. Comparative analysis of Aminoacid profile of three polychaete species (per 100 grams of each sample)](image)

The results of the amino acids profile (Fig. 2) in three polychaete species was reported with 10 essential amino acids (EAA) and 10 non-essential amino acids (NEEA). The maximum essential amino acids content was recorded (EAA) in *N. virens* (5.2716 g) followed by *P. cultrifera* (4.7009 g), and *Glycera* sp (4.1204 g) and the maximum NEAA content was recorded in *P. cultrifera* (4.4212 g), followed by *Glycera* sp (3.1265 g) and *N. virens* (1.8041 g). In *P. cultrifera*, the maximum content of EAA was reported with lysine (1.1356 g) and minimum value with histidine (0.1095 g) and the maximum content of NEAA was reported with alanine (1.1245 g) and minimum content with tyrosine (0.1195 g); in *N. virens*, EAA reported maximum with lysine (1.9347 g) and minimum with leucine (0.1895 g) and NEAA reported maximum with aspartic acid (0.3456 g) and minimum with serine (0.0784 g) and *Glycera* sp, EAA reported maximum with lysine (1.2057 g) and minimum with methionine (0.1903) and NEAA reported maximum with aspartic acid (0.8457 g) and minimum value of proline (0.1193 g).
In this study, EAA was recorded higher content when it compared to NEAA in all the three species of polychaete. In the EAA the most dominant amino acids are lysine, arginine, and isoleucine and in the NEAA are cysteine, alanine, asparagine, and aspartic acid whereas the lesser EAA and NEAA are methionine and serine, respectively. This similar observation has been reported in *P. cultrifera* (Dorham *et al*., 2015) and in mariculture wastewater polychaete, *P. helleri* (Palmer *et al*., 2014). Essential amino acid like leucine can stimulate the protein synthesis was reported by Volek, (2009) and the lysine and tyrosine have essential roles in reproductive functions and nutrition of developing gametes (Fischer *et al*., 1996; Hoeger and Abe, 2004). On the other hand, the NEAAs such as alanine, proline, and glycine are considered as osmolytes in *P. cultrifera* (Jeuniaux *et al*., 1961) and in other polychaetes and marine invertebrates (Hoeger and Abe, 2004, Kube *et al*. 2007). Alternatively, serine is involved in methyl group transfer, which is responsible for different processes in the cell and contributes to the formation of S-adenosylmethionine, the methyl donor (Amelio *et al*., 2014).

Three species of polychaetes showed difference among vitamins (Fig. 4) composition: Vitamin B1 (Thiamine) content was maximum in *P. cultrifera* (1.55 mg) followed by *Glycera* sp (1.34 mg), and *N. virens* (in traces); Vitamin B2 (Riboflavin) content was maximum in *P. cultrifera* (1.29 mg) followed by *N. virens* (0.62 mg) and *Glycera* sp (0.45 mg); Vitamin B3 (Niacinamide) level was maximum in *P. cultrifera* (1.34 mg) followed by *N. virens* (0.45 mg) and *Glycera* sp (0.34 mg) about; Vitamin B6 (Pyridoxamine) level was maximum in *Glycera* sp (1.56 mg) followed by *P. cultrifera* (0.50 mg) and *N. virens* (0.10 mg); Vitamin B9 (Folic acid) content was maximum in *N. virens* (0.003) followed by *Glycera* sp (0.002 mg) and *P. cultrifera* (0.001); Vitamin B12 (Cyanocobalamin) content was maximum in *Glycera* sp (1.22 mg), whereas *P. cultrifera* and *N. virens* in traces; Vitamin A content was maximum in *P. cultrifera* (0.357 mg) followed by *N. virens* (0.017 mg) and *Glycera* sp (0.016 mg); likewise, Vitamin E content was maximum in *P. cultrifera* (3.88 mg) followed by *N. virens* (2.91 mg) and *Glycera* sp (1.33 mg); and finally, Vitamin C reported with maximum in *N. virens* (20.1 mg) followed by *Glycera* sp (11.9 mg) and *P. cultrifera* (0.78 mg). Not much study has been conducted on vitamin and mineral content in polychaete. In vitamin study, *P. cultrifera, N. virens* and *Glycera* sp showed higher content of vitamins A, B1, and E and less in vitamin C and this similar observation has been made in *P. nuntia* (Techaprempreecha *et al*., 2011).
The fat-soluble vitamins A, D, and E are essential to support the growth of the shrimp (He et al., 1992). Dietary levels of thiamin (B1), riboflavin (B2), niacin, vitamin B6, vitamin B12, choline, inositol, and ascorbic acid were also recommended for the maximum growth of the several species of shrimp (D’Abramo and Conklin, 1992). Earlier studies have been conducted in shrimp with vitamin E showed that the improvement of the percentage of normal rate of sperm and ovarian maturation (Chamberlain, 1988), whereas Alava et al., (1993b) proved that the ovarian maturation was retarded when the deficiency of any one of these vitamins like E, A, and C. In the present study, all the three polychaete species were recorded with required quantity of vitamin as essential nutrition for shrimp aquaculture.

The mineral (Fig. 5) composition varied among the three polychaete species, each of 100 grams: Calcium was maximum in Glycera sp (84.3 mg) followed by N. virens (56.7 mg) and P. cultrifera (25.6 mg); likewise, Magnesium content was maximum in Glycera sp (25.7 mg) followed by N. virens (17.32 mg) and P. cultrifera (12.9 mg); Zinc content varied from species as N. virens (1.79 mg), Glycera sp (1.55 mg), and P. cultrifera (1.13 mg); Iron content showed maximum in P. cultrifera (4.21 mg) followed by Glycera sp (3.46 mg) and N. virens (1.90 mg); Sodium content varied maximum in N. virens (156.70 mg), P. cultrifera (147.50 mg), and Glycera sp (145.20 mg); Potassium was maximum in Glycera sp (201.30 mg) followed by P. cultrifera (94.30 mg) and N. virens (90.30 mg); and Sodium level was maximum in N. virens (1456.70 mg) followed by Glycera sp (894.60 mg) and P. cultrifera (456.60 mg). Mineral content was dominant with chloride of all the three species of polychaete and this similar study has been made in P. nuntia (Techaprempreecha et al., 2011). According to Harrison (1990) that the mineral deficiencies or imbalances could be affected the crustacean reproduction negatively and the physiological stresses could be trigger the oocyte resorption or reduce the reproductive fitness of the broodstock. Additionally, the minerals malnutrition are could cause the altered composition and the quality of eggs. However, the studies on mineral requirements for shrimp brood stock was very less and in most of the studies the artificial diets the mixer of the minerals were formulated, especially to fortify with calcium, phosphorus, magnesium, sodium, iron, manganese, and selenium (Chamberlain, 1988; Alava et al., 1993 a, b; Marsden et al., 1997; Mendoza et al., 1997; Xu et al., 1994).
Fig. 5. Comparative analysis of Mineral content of three polychaete species (per 100 grams of each sample)

From the earlier studies, it was evidently understood that nutrition is an important factor in growth and breeding of penaeid shrimps, and yet, knowing shrimp nutrition requirements is essential for successful management of broodstocks (Alireza Salarzadeh, 2014). The polychaetes are likely to become increasingly important as a resource in relation to the development of aquaculture for crustacea (Milada Safarik, 2007) since it has been found that polychaetes can provide a nutritionally correct balance of polyunsaturated fatty acids, which are essential for egg maturation in cultured prawns (Lytle et al., 1990) or can provide other factors are essential for egg maturation (Croz et al., 1990). In addition, polychaete worms may cause better absorption of chemical compositions like cholesterol and long chain unsaturated fatty acids (HUFA), which are effective for growth of ovary and production of eggs with high quality (Alireza Salarzadeh, 2014) and also diets that lack essential fatty acids delay shrimp growth (Millinema et al., 1986, Sudaryono et al., 1995). 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 (Palmer et al. 2014). This constraint arises because the most valuable cultured crustaceans and fish all require polyunsaturated fatty acids (PUFA) in their diet (Izquierdo et al. 2001), especially for the production of viable young ones. However, the nutritional value of polychaetes as well as other diets can vary with species, season of harvest and life stage (Chimsung, 2014).

ACKNOWLEDGEMENTS

The authors express their thankfulness to the financial support for this research work under RUSA-Phase 2.0 scheme, Govt of India, Department of Oceanography and Coastal Area Studies, Alagappa University, Karaikudi.

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Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), 8(X), 2020: http://dx.doi.org/10.7770/safer-V0N0-art2188


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Received: 30th April 2020; Accepted: 07th August 2020; Published: 14th September 2020.