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Impact of fishmeal replacement with *Arthrospira platensis* on growth performance, body composition and digestive enzyme activities of the freshwater prawn, *Macrobrachium rosenbergii*

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ABSTRACT

In this study, we assessed the suitable level of replacement of fishmeal with a blue green microalga, *Arthrospira platensis* in feed for the post larvae (PL) of the freshwater prawn, *Macrobrachium rosenbergii* by evaluating the growth performance, prawn proximate composition, feed utilization parameters and the activity of digestive enzymes. The prawns were fed 5 different diets: a control diet and 4 different diets containing *A. platensis* at various levels such as 25%, 50%, 75%, 100%. These diets were fed to the PL for 90 days in triplicates. The growth performance in terms of weight gain, specific growth rate and feed efficiency ratio were found significantly ($P < 0.05$) higher in the prawns fed with the 50% of *A. platensis* feed fed group. At this level of 50% replacement, the prawn proximate composition, such as total protein, amino acids, carbohydrates and lipid contents were significantly ($P < 0.05$) higher than the control. Similarly, feed utilization parameters, such as feeding rate, absorption rate and conversion rate were significantly ($P < 0.05$) higher than the control. Added to that, the activity of the digestive enzymes such as protease, amylase and lipase showed the same trend at the level of 50% replacement. The hierarchy of the growth performance in prawns corresponds to 50 > 25 > 75 > 100% replacement of fishmeal with *A. platensis*. These results concluded that a partial replacement of the fishmeal with *A. platensis* at the level of 50% is beneficial for the growth of prawn *M. rosenbergii*.

1. Introduction

The giant freshwater prawn, *M acrobrachium rosenbergii* known as 'scampi' in commercial parlance, is a highly valued delicious food and commands a very good demand in both domestic and export market. *M. rosenbergii* culture is gradually gaining momentum in the present era owing to its price, taste, fast growth rate, less susceptibility to diseases and its compatibility to grow with carps (New, 2005; Radheyshyam, 2009). It has a wide distribution throughout the Indo-Pacific region and most favoured for farming in tropical and subtropical areas of the world (New, 2002, 2005). This freshwater prawn is farmed chiefly in small to medium-sized earthen ponds in West Bengal, Andhra Pradesh, Tamil Nadu and Kerala in India (Nair and Salin, 2012). Chand et al., 2015 reported and suggested that salinity plays a significant role in the culture of *M. rosenbergii* and the species showed satisfactory growth and survival at wide salinity range (0–15 ppt) and *M. rosenbergii* can be considered as an ideal species to promote. Global production of this prawn has increased from 130,689 t in 2000 to 203,211 t in 2011 (FAO, 2013). The total scampi production from India in 2010–2011 was about 8778 metric tonnes and West Bengal was the leading producer. In 2011–12, India exported 2723 metric tonnes *M. rosenbergii* with an increase of 31.61% than the previous years (MPEDA, 2011).

Paralleling the growth of the aquaculture industry, there has been an expansion in feed production (Tacon and Savas, 2000). Fishmeal (FM) as well as other marine meals are often included in the aquatic feeds as they are considered to be the excellent sources of high quality proteins, highly unsaturated fatty acids (HUFA), vitamins, minerals and attractants (Tacon and Akiyama, 1997; Gatlin et al., 2007; Tacon and Metian, 2008; Naylor et al., 2009). Due to these properties, FM has become one of the primary components of commercial feed formulations. Although, worldwide FM produc-

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tion has attained a relatively stable level, it still could not match the rapid worldwide development of aquaculture (Goytortua-Bores et al., 2006). Due to the limited supply of fishmeal and oil from wild catches, the efficient use and sharing of these products is a major issue for the aquaculture industry (Kaushik and Troell, 2010; Tacon and Metian, 2009). The fish in-fish out ratio (FIFO) is the efficiency at which the aquaculture converts a weight-equivalent unit of wild fish into a unit of cultured fish. Aquaculture converts 65% of the wild fish into fishmeal at a FIFO ratio of between 0.66 (Jackson, 2009; Kaushik and Troell, 2010) and 0.7 (Tacon and Metian, 2008). However, as a result of decreasing the supply of fishery byproducts and various concerns over its quality, the aquaculture industry is now actively investigating the alternative protein sources to be added to the feed.

The need for alternative protein sources to fishmeal has brought attention to many products that are local or regional in nature (Nyina-wamwiza et al., 2012), including single cell proteins, such as yeasts, bacteria, and microalgae (Mukhopadhyay and Ray, 1999; Ng et al., 2002; Skrede et al., 2002; Bairagi et al., 2004; Refstie et al., 2005; Hemaiswarya et al., 2011). In order to be used in aquaculture, a micro algal strain has to meet various criteria, such as ease of culturing, lack of toxicity, high nutritional value with correct cell size and shape and a digestible cell wall to make nutrients available (Raja et al., 2004; Patil et al., 2007). Protein and vitamin content is a major factor determining the nutritional value of microalgae. In addition to it, Polyunsaturated fatty acids (PUFA) e.g. eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA) content is of major importance for an aquaculture organism. Different strategies were being practiced to improve the polyunsaturated fatty acids content in microalgae (Guedes et al., 2010). Also, the crude protein extracts of microalgae rich in amino acids, including the essential amino acids (EAA) isoleucine, leucine, lysine, methionine, phenylalanine, and valine (Becker, 2007; Uslu et al., 2011; Mahboob et al., 2012). It is often advised to use mixed microalgae cultures in order to have a good protein profile, adequate vitamin content and high polyunsaturated fattyacids, mainly EPA, AA and DHA, as they are considered to be essential for the survival and growth during the early stages of life of many marine animals (Volkman et al., 1989). One of the beneficial effects attributed to adding algae is an increase in ingestion rates of food by marine fish larvae which enhance the growth and survival as well as the quality of the larvae of *Hippoglossus hippoglossus* (Naas et al., 1992). In addition, the presence of algae in rearing tanks of European sea bass larvae has been shown to increase the digestive enzyme secretion (Cahu et al., 1998). The green algae *Haematococcus pluvialis,* (Yuang and Chen, 2000), *Chlorella zofingiensis* (Bar et al., 1995) and *C. vulgaris* (Gouveia et al., 2003; Gouveia and Rema, 2005) are used as dietary carotenoid sources for fish and shrimp species. The marine algae *Nanofrustulum* (MAP) and *Tetraselmis* (MAP8) used as alternative feed source and noted the better improvement in the body carcass composition on Atlantic Salmon (*Salmo salar*), common carp (*Cyprinus carpi*) and white leg shrimp (*Litopenaeus vannamei*) (Kiron et al., 2012). Also, the fishmeal replacement with *A. platensis*, *C. vulgaris* and *Azolla pinnata* diet improved the antioxidant status in *M. rosenbergii* post larvae (Radhakrishnan et al., 2014).

Spirulina, (*A. platensis*) is a blue-green alga (Cyanobacterium, family Oscillatoriaceae), which is gaining more attention from medical scientists as a nutraceutical and source of potential pharmaceuticals (Kapoor and Mehta, 1994). In 1974, the United Nations World food conference declared *Arthrospira* as "the best for tomorrow" and it has gained popularity in recent years as a food supplement (UNWFC, 1975). Basically, *Arthrospira* consists of 55–70% protein and 5–6% lipid (w/w dried cell). Polyunsaturated fatty acids (PUFAs) constitute 1.5–2% of the total lipid content of this alga. In fact, it is rich in γ-linolenic acid (36% of the total PUFAs), vitamins, essential minerals, antioxidants, carotenoid pigments and enzymes (e.g. lipase) (Belay, 2002; Desai and Sivakami 2007; Demir and Tukel, 2010). Also, *Arthrospira* induces the activity of immune system. It builds up both the cellular and humoral arms of the immune systems and thus improving their ability to function inspite of stresses from environmental toxins and infectious agents (Hayashi et al., 1994; Qureshi et al., 1995), natural biochelated vitamins, especially β-carotene (Seshadri et al., 1991) and an antioxidant enzyme superoxide dismutase (SOD), *S. fusiformis* possess potent antiviral activity (Hayashi et al., 1996), anti cancer effects (Mittal et al., 1999), strengthens immune system (Qureshi et al., 1995, 1996) and significantly reduce the nephrotoxicity (Sharma et al., 2007). In aquaculture, The administration of hot-water extract of *A. platensis* via injection, and immersion routes was reported to enhance the immunity of white shrimp *L. vannamei* and its resistance to *V. alginolyticus* and environmental stress (Tayag et al., 2010; Lin et al., 2010), regulate the antioxidant status in *M. rosenbergii* and *L. vannamei* (Lin et al., 2010; Radhakrishnan et al., 2014), increase the carotenoids and heamato-immunological parameters decrease the stress in *Oncorhynchus mykiss* (Teimouri et al., 2013; Yeganeh et al., 2015) and improve the health status of *Oreochromis niloticus* (Ibrahem and Ibrahim, 2014). Therefore, the biomass of this rich source of nutrients play a vital role in feed and food additives in the agriculture, food, pharmaceuticals, and perfumery industries (Hoseini et al., 2013). The present study was conducted to evaluate the suitable level of replacement of fishmeal with *A. platensis* in diets fed to *M. rosenbergii* postlarvae (PL), and to assess the growth promotion, nutritional and energy utilization parameters, biochemical constituents and digestive capability of *M. rosenbergii* PL.

2. Materials and methods

2.1. Prawns

The postlarvae of *M. rosenbergii* (PL 15) were purchased from the Government prawn hatchery (Thrissur, Kerala) and were safely brought to the laboratory in well-oxygenated plastic bags. They were stocked in a large cement tank ($1.83 \times 1.22 \times 0.91$ m) and were allowed to acclimatize for 2 weeks to the laboratory conditions. During this period, the prawns were fed with boiled egg albumin (egg custard), *Artemia nauplii* and crumble feed (purchased from Rosen fisheries, Thrissur, Kerala) alternatively twice a day. The animals were maintained at natural photoperiods and the room temperature was maintained at $25\,^{\circ}C \pm 2\,^{\circ}C$.The maintenance procedures such as removal of excreta and unused feed, renewal of three fourth of the water were conducted daily. The acclimatization tank was adequately aerated.

2.2. Arthrospira platensis *culture*

The *A. platensis* pure culture was collected from *Spirulina* production research and training centre Kadachanendal, Madurai, Tamil Nadu, India.

2.2.1. *Inoculation of* A. platensis

Inoculation of the microalgae *A. platensis* was done by adding 100 ml of the microalgae mother culture to 900 ml of *Spirulina* medium (Schlosser, 1994) and the cultures were incubated for 15 days at $24 \pm 1\,^{\circ}C$ in a thermo-statically controlled room. The room was illuminated with cool inflorescence lamps (Phillips 40 W, cool daylight 6500 K) at an intensity of 2000 lux in a 12:12 h light dark regime.