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RESEARCH PAPER

Improving the quality of tiger shrimp *Penaeus monodon* through dietary incorporation of algae as a source of natural pigment

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تحسين نوعية روبيان النمر (*Penaeus monodon*) بإستخدام الطحلب كمصدر طبيعي للصبغة في الغذاء

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Abstract. Tiger shrimp is one of the major candidate species for export oriented aquaculture which dominates the seafood market in regions of European Union, Japan and USA. Carotenoid content in seafood has now become one of the important criteria in determining the quality of edible product. Recent trends in supplementing fish diets with natural pigment source are an alternative to the utilization of expensive synthetic pigments. In this context, green algae *Enteromorpha intestinalis* was selected as a source of natural pigment for inclusion in the diet of tiger shrimp *Penaeus monodon*. Astaxanthin being an important category of carotenoid pigment was monitored in shrimp muscle tissue during the feeding trial. Significant variation ($p<0.05$) was observed between the experimental groups as confirmed through ANOVA thus exhibiting higher astaxanthin content of shrimps (18.70 ± 4.48 ppm) fed with *E. intestinalis* incorporated diet as compared to control (15.80 ± 2.33 ppm). The present programme therefore emphasizes on the quality improvement of aquaculture product by dietary inclusion of algae as a natural pigment source.

Keywords: Shrimp; aquaculture, feed, algae, pigment.

المستخلص: يعتبر روبيان النمر أحد أهم الأنواع المستزرعة للتصدير التي تهيمن على سوق المأكولات البحرية في مناطق الاتحاد الأوروبي واليابان والولايات المتحدة الأمريكية، وحالياً يعتبر محتوى الكاروتين (Carotenoid) الموجود في المأكولات البحرية أحد أهم المعايير المستخدمة في تحديد جودة المنتجات الصالحة للاستهلاك. ويتم التوجه حالياً لاستخدام الصبغات الطبيعية كبديل للصبغات الاصطناعية عالية التكلفة المستخدمة في الغذاء السمكي، وفي هذا السياق تم اختيار الطحلب الأخضر (*Enteromorpha intestinalis*) كمصدر طبيعي للصبغة لإدراجها في الغذاء المستخدم لروبيان النمر (*Penaeus monodon*). تمت متابعة نسبة الأستاكسانثين (Astaxanthin) والذي يعتبر أحد اهم فئات الكاريتينويد (Carotenoid) في الأنسجة العضلية للروبيان خلال تجارب التغذية، وقد لوحظ وجود اختلاف هام ($p < ٠,٠٥$) بين المجموعات التجريبية كما أثبته اختبار تحليل التباين (ANOVA) والذي يظهر وجود محتويات أعلى لـ Astaxanthin (١٨,٧٠ ± ٤,٤٨ جزء في المليون) في الروبيان الذي تغذى على وجبات تحتوي على *E. intestinalis* مقارنة مع الروبيان المتحكم في غذائه (١٥,٨٠ ± ٢,٣٣ جزء في المليون)، وبالتالي فإن البرنامج الذي تم استخدامه يثبت تحسن جودة منتجات الكائنات البحرية المستزرعة من خلال إدراج الطحالب كمصدر طبيعي للصبغة في غذائها.

الكلمات المفتاحية: الربيان، الاستزراع السمكي، أعلاف الأحياء المائية، الطحالب، الصبغة

Introduction

Inclusion of algae as dietary supplement in animals have been investigated previously as a source of pigment (Strand *et al.*, 1998). The effects of dietary inclusion of algae have resulted in improved performance including better animal product quality (Moss, 1994; Penaflorida and Golez, 1996; Cruz-Suarez *et al.*, 2000). To be more specific the commercial production of shrimps and prawns as an edible food product represents one of the fastest growing areas of aquaculture (Rosenberry, 2005) with high consumer appeal and attractive market for shrimps due to their body colouration or pigmentation which is a direct measure of its astaxanthin content. Carotenoid utilization by aquaculture species is well documented as it plays a regulatory role in providing antioxidant and pro-vitamin A activity, enhancing immune response, improving reproductive performance, growth, maturation and photo-protection (Howell and Matthews, 1991). They also help the species to resist environmental stress (Meyers, 1994). Algae and higher groups of plants are the major producers of carotenoids which comprises a family of over 600 natural fat soluble pigments (Britton *et al.*, 1981). Studies reveal that alternative utilization of plant pigments in formulated diets have improved the body pigmentation

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Figure 1. Map showing the *P. monodon* farming site at Bali Island of Indian Sundarbans.

of farmed crustaceans, particularly penaeids in order to achieve better market price (Lorenz, 1998; Liao and Chien, 1994).

Therefore the present work is an attempt to utilize *Enteromorpha intestinalis* as a natural dietary pigment source for farmed tiger shrimp (*Penaeus monodon*) in relation to its quality improvement.

Materials and methods

Collection of algae and preparation of experimental diets

Live and healthy algae *Enteromorpha intestinalis* was collected from Bali Island (22° 04′ 35.17″ N latitude and 88° 44′ 55.70″ E longitude) of Indian Sundarbans during low tide. The collected material was rinsed in ambient water and then with distilled water, oven-dried at 50°C and finally processed to make powder. Experimental diet was formulated through incorporation of algae ($Diet_{ENT}$) at a level of 5%. Simultaneously a control diet ($Diet_{C}$) was also formulated to study the comparative performance (Table 1).

Feeding trial

A feeding trial was run at Bali Island (22°04′ 35.17″N latitude and 88° 44′ 55.70″ E longitude) of Sundarbans in grow-out ponds for 90 days of experimental duration (Fig. 1). Shrimp juveniles were procured from hatchery and stocked in experimental ponds at a density of 2 individuals/m^2. Experimental diets were randomly assigned, the culture species were fed twice daily and the uneaten feed was checked at regular intervals.

Table 1. Formulation of experimental diets.

Ingredients	Diet $_{Control}$	Diet $_{Enteromorpha}$
Fish meal	35	30
Soybean oil cake	11	11
Mustard oil cake	11	11
Rice polish	23	23
Wheat flour	16	16
oyster shell dust	2	2
Shark oil	2	2
Enteromorpha intestinalis (source of astaxanthin)	0	5

Astaxanthin analysis

The astaxanthin content in shrimp muscle tissue was analyzed according to the spectrophotometric method outlined by Schuep and Schierle (1995). Its value in % was converted to ppm level for easy interpretation of data. The body colouration of shrimps after boiling was compared by Roche SalmoFanTM colour score.

Statistical analysis

The collected data were finally subjected to one-way analysis of variance (ANOVA). All statistical calculations were performed with SPSS 9.0 for Windows.

Results and discussion

The average astaxanthin content in muscle tissue was higher in shrimps fed with DietENT as compared to DietC (Table 2). A darker orange-red colouration was observed in shrimps fed with DietENT after boiling them in water for 5 minutes when compared with Roche SalmoFanTM colour score. The colour score was 30 for DietENT fed shrimps whereas a score of 27 was recorded from shrimps fed with DietC. ANOVA results showed significant variation ($p<0.05$) in average astaxanthin content which may be attributed to the capability of P. monodon to easily convert the fraction of algal astaxanthin into tissue astaxanthin. The *Enteromorpha*

Table 2. Variations in astaxanthin content of *P. monodon* (ppm muscle tissue) fed with experimental diets.

Days of culture (DOC)	Diet $_{Control}$	Diet $_{Enteromorpha}$
0	12.83 ± 0.22	13.02 ± 0.27
30	15.21 ± 0.22	17.62 ± 0.55
60	16.96 ± 0.53	20.71 ± 0.76
90	18.22 ± 1.23	23.47 ± 1.31
Average	**15.80 ± 2.33b**	**18.70 ± 4.48a**

**Means with different letters(a,b) in a row differ significantly ($p<0.05$); values are means of three replicates*

sample selected for the investigation is found to contain 120.78 ppm astaxanthin as reported from the present study region (Mitra *et al.*, 2013; Banerjee *et al.*, 2009; Chakraborty and Santra, 2008). Carotenoid, particularly astaxanthin content of feed is one of the major factors influencing the colour development in animals (Moretti *et al.*, 2006) but at the same time scientific knowledge about several factors like dietary pigment source, their dosage level, feeding duration, dietary composition and magnitude of carotenoid esterification is also required to identify these interaction processes (Meyers and Latscha, 1997; Bjerkeng, 2000; Buttle *et al.*, 2001; Gomes *et al.*, 2002; White *et al.*, 2002).

The present study showed significantly different astaxanthin content of the farmed shrimp which are in agreement to the observations that crustaceans exhibit strong tendency towards selection of specific carotenoids at a specific rate for their metabolic absorption (Meyers and Latscha, 1997). Similar work conducted from the present study region reveal that *P. monodon* when fed with diet containing red algae *Catenella repens* at a level of 5% improved the body astaxanthin content (Banerjee *et al.*, 2010). The search for natural astaxanthin was not only limited to the algal resources, rather salt-marsh grass *Porteresia coarctata* was also tested as a natural dietary astaxanthin source in *P. monodon* feed with better results from the present geographical locale (Mitra *et al.*, 2011, 2013). In continuation such natural carotenoid supply to the diet of shrimps has been studied for *P. japonicus* and *Litopenaeus vannamei* too from different parts of the globe. The ingredients of natural origin that have been used in the diet are red yeast (*Phaffia rhodozyma*) and microalgae *Dunaliella salina* (Chien and Jeng, 1992); *Chnoospora minima* (Menasveta *et al.*, 1993); *Spirulina sp.* (Liao *et al.*, 1993; Chien and Shiau, 1998); *Haematococcus pluvialis* (Chien and Shiau, 1998) and *Isochrysis galbana* (Pan *et al.*, 2001). An usual trend of marked increase in the body carotenoid content has been observed when organisms were fed with plant pigment source diets. For example, feed supplemented with 50 ppm algal material (*Dunaliella salina*) improved the body colouration of *P. monodon* (Boonyaratpalin *et al.*, 2001). Three types of diet when provided to *P. semisulcatus* containing natural carotenoid sources like red pepper and marigold flower resulted in higher carotenoid accumulation in body tissues (Gocer *et al.*, 2006). However research findings from Mexico also reported that feed incorporated with cultivated green alga *Ulva clathrata* significantly improved the body pigmentation of farmed shrimp *L. vannamei* (Cruz-Suarez *et al.*, 2009).

Conclusions

Improved product quality of farmed *P. monodon* clearly reflects the transforming potential of algal astaxanthin into the body tissues by the particular culture species. Thus the present study provides a baseline information about the natural astaxanthin pool of the Indian Sundarbans which may serve as an alternative to synthetic astaxanthin in animal diets which are more expensive.

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