



Research Article



Dietary Supplementation of *Dunaliella Salina* on Growth Performance and Body Composition of Indian White Shrimp, *Fenneropenaeus Indicus* (H. Milne Edwards)



Osamah A Ahmad, Adnan J Salama, Sambhu Chithambaran*

Department of Marine Biology, Faculty of Marine Science, King Abdulaziz University, Jeddah, Saudi Arabia

*Corresponding Author: Chithambaran, S. Department of Marine Biology, Faculty of Marine Science, King Abdulaziz University, Jeddah, Saudi Arabia. Tel: +966552790434; E-mail: sambhu@kau.edu.sa

Abstract

Juveniles of white shrimp, *Fenneropenaeus indicus* were fed with a micro green alga, *Dunaliella salina* through a fishmeal based supplementary diet containing 35% protein for a period of 180 days in fiberglass tanks. There were control and treatments for the study and both were triplicated. *D. salina* was incorporated at 0.5, 1.0, 2.0, 4.0 and 8.0% in the diet and was designated as treatments T1, T2, T3, T4 and T5 respectively. *D. salina* free diet was considered as Control. The diet containing *D. salina* at 2.0% (T3) showed significantly ($p < 0.05$) superior growth when compared to control and other treatments. Protein efficiency ratio and protein digestibility were found to be high in T3 and feed conversion efficiency was reduced. Better colour and taste were observed in *D. salina* fed shrimp compared to control. Crude protein, lipid, ash and nitrogen free extract (NFE) of the carcass did not show significant difference between control and treatments. Dietary administration of *D. salina* enhances growth and body colour in white shrimp *F. indicus*.

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Introduction

The production of natural feed additives for the aquaculture industry is a thriving sector. These substances are being used for several purposes including the enhancement of the immune systems of farmed shrimp, promoting growth, attaining the desired flesh and skin pigmentation, as well as improving the organoleptic properties of the farmed product^[1,2]. Astaxanthin is the major carotenoid responsible for the pink-red pigmentation of many fish and shrimp species^[3]. Carotenoids are a source of pro-vitamin A which increase survival rate, weight gain and disease resistance against white spot syndrome virus in shrimp^[4,5]. Carotenoids not only control ammonia level and resistance to stress but also improve hepato-pancreatic function, cholesterol and polyunsaturated fatty acids from oxidation and provide antioxidant protection^[6-8]. Among the carotenoids, astaxanthin was found to be more effective than β -carotene as an antioxidant and a better agent to destroy free radicals than other carotenoids^[9,10].

Dunaliella salina is a microalgae occurring naturally in a number of locations worldwide. In the marine environment, *D. salina* appears green, however, in conditions of high salinity and light intensity, the microalgae turns red due to the production of protective carotenoids in the cells^[11]. The majority of harvested micro-algae are currently being sourced from one of the most pristine environments in

the world – a remote coastal salt lagoon in Western Australia^[12]. *D. salina* is harvested without any harmful solvents or chemicals and the carotenoids (highly-prized anti-oxidant pigments responsible for the red color) are then extracted for use in pharmaceuticals, cosmetics, nutritional supplements, aquaculture feeds and food coloring^[13]. *D. salina* paste has a wide range of applications as feed stock. As a direct feed for filter feeders such as corals and sponges, as an enrichment for live feed organisms such as *Artemia* and *Rotifers*, especially for marine ornamental fish where the coloration is an important consideration, as an enrichment in fresh food (Mussels, Pipis, etc.) for crustacean larvae and brood stock, as a natural source of carotenoids in diets for shrimps and fish^[14,15]. Considering the importance of *D. salina* as a feed additive in aquaculture, the present study was conducted to evaluate the effect of *D. salina* on growth, pigmentation and body composition in Indian white



shrimp, *Fenneropenaeus indicus* an ideal candidate species for coastal aquaculture practice in the Kingdom.

Results

Growth Performance

Data on growth performance of shrimp are presented in Table 1 and Figure 1. A diet containing *D. salina* at 2.0% (T3) showed superior growth when compared to control and other treatments. ANOVA shows that there is significant difference ($p < 0.05$) on weight between control and treatments. High SGR (%) was observed in T3 followed by T4, T2 and T1 and it was low in T5. Biomass was high in T3 compared to control and other treatments. Highest survival was recorded in Control followed by T1, T3, and T4 and it was lowest in T2.

Table 1: Growth, survival and biomass of shrimp fed *D. salina* diets

Parameters	Control M±SD	T1 M±SD	T2 M±SD	T3 M±SD	T4 M±SD	T5 M±SD
Initial length (cm)	9.2±0.1	9.2±0.1	9.2±0.1	9.2±0.1	9.2±0.1	9.2±0.1
Initial weight (g)	4.4±0.1	4.4±0.1	4.4±0.1	4.4±0.1	4.4±0.1	4.4±0.1
Final length (cm)	13.6±0.9	13.6±0.7	13.5±0.8	14.3±0.7	14.2±0.6	13.3±0.9
Final weight (g)*	15.5±3.6 ^a	16.1±2.8 ^a	16.7±2.8 ^{ab}	17.6±2.9 ^b	17.2±3.4 ^b	15.1±3.0 ^a
Net length gain (cm)	4.4±0.6	4.4±0.9	4.3±0.8	5.1±0.5	5.0±0.6	4.1±0.9
Net weight gain (g)	11.1±1.6	11.7±1.2	12.3±0.9	13.2±1.3	12.8±31.7	10.7±1.0
SGR (%)	0.83±0.3	0.85±0.3	0.85±0.4	0.89±0.3	0.88±0.4	0.82±0.3
Survival (%)*	78.3±2.6 ^d	63.3±3.1 ^c	48.3±2.9 ^a	60±2.0 ^c	58.5±2.8 ^b	58.3±4.0 ^b
Biomass (g)	730.3±12.1	610.6±9.8	471.4±10.3	632.0±13.2	600.4±11.1	530.1±9.4

M-Mean; SD- Standard Deviation

* $p < 0.05$; a, b, c, d. Means with the same superscript do not differ from each other (Duncan's test)

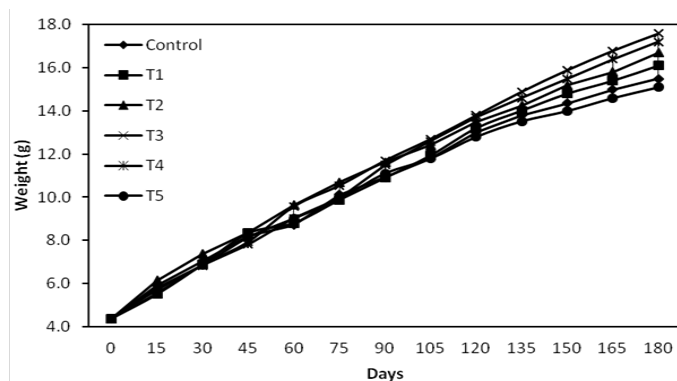


Figure 1: Growth performance of *F. indicus* fed *D. salina* diets

Water Quality Parameters

Data on physical and chemical water quality parameters observed during the culture period is depicted in Figure 2, 3. These parameters were found to be within the range suitable for

shrimp growth and there was no significant difference ($p > 0.05$) on parameters between control and treatment tanks.

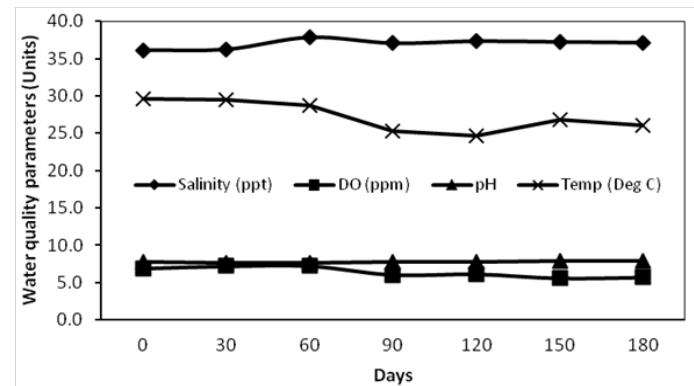


Figure 2: Physical water quality parameters during the culture period

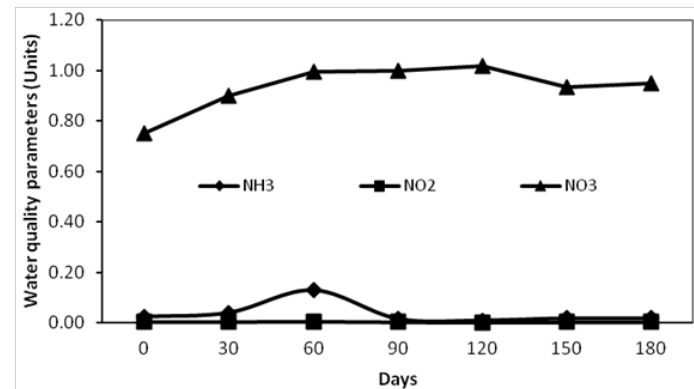


Figure 3: Chemical water quality parameters during the culture period

Feed Utilization

Details of feed utilization study are presented in Table 2. Protein Efficiency ratio and apparent protein digestibility were found to be high in T3 when compared to control and other treatments. Feed conversion ratio was low in T3. Significant difference ($p < 0.05$) in feed conversion efficiency was observed between control and T3 but there was no significant difference in feed consumption, excretion, assimilation and protein digestibility between control and treatments.

Table 2: Feed utilization of shrimp fed *D. salina* diets

Parameters	Control M ±SD	T1 M ±SD	T2 M ±SD	T3 M ±SD	T4 M ±SD	T5 M ±SD
Feed consumption (g) ^{NS}	1±0.0	1±0.0	1±0.0	1±0.0	1±0.0	1±0.0
Excretion (g) ^{NS}	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.0
Assimilation (g) ^{NS}	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1
FCE (%) [*]	2.90±0.3 ^c	2.40±0.4 ^b	2.20±0.3 ^b	1.80±0.5 ^a	2.20±0.3 ^b	2.10±0.4 ^a
Protein efficiency ratio ^{NS}	2.57±0.7	2.28±0.6	2.28±0.6	3.14±0.7	2.85±0.6	2.00±0.4
Protein digestibility (%) ^{NS}	78.9±1.8	79.4±1.5	80.0±2.2	82.0±2.6	81.1±1.8	78.9±1.7

* $p < 0.05$; NS- $p > 0.05$; M- Mean; DS- Standard Deviation; FCE-Feed conversion efficiency

a, b, c. Means with the same superscript do not differ from each other (Duncan's test)

Physical Quality of Shrimp

Details on physical quality of shrimp after culture are presented in Table 3. Soft shell and loose shell percentage was low in *D. salina* fed shrimps compared to control and the observed difference between control and treatments was significant ($p < 0.01$). Shrimp colour and taste were found to be increased in higher dose of *D. salina* fed shrimps.

Table 3: Physical quality of shrimp fed *D. salina* diets

Parameters	Control M ±SD	T1 M ±SD	T2 M ±SD	T3 M ±SD	T4 M ±SD	T5 M ±SD
Soft Shell (%)*	4.2± 0.6	3.0 ± 0.3	6.9± 2.1	5.6± 1.1	2.9± 0.9	5.0± 2.1
Loose Shell (%)*	27.7± 2.3 ^c	20.7± 3.1 ^a	24.1± 1.9 ^b	22.2± 2.5 ^b	20.0± 2.8 ^a	20.0± 1.8 ^a
Hard Shell (%)*	68.1± 2.5 ^a	76.3± 3.4 ^b	69.0± 3.6 ^a	72.2± 2.6 ^b	77.1± 1.6 ^b	75.0± 2.9 ^b
Shrimp colour (cooked)	light	light	light	Dark	Dark	Dark
Taste	Bland	Bland	Light sweet	Light sweet	Light sweet	Light sweet

* $p < 0.05$; M- Mean; DS- Standard Deviation

^{a, b, c}. Means with the same superscript do not differ from each other (Duncan's test)

β-Carotene Analysis

Data on β-carotene analysis is shown in Table 4. The average β-carotene level in *D. salina* is found to be 0.5%. The residual β-carotene content in shrimp carcass after experiment is found to be negligible.

Table 4: β-carotene content in *D. salina*, feeds and carcass

Parameters	Control	T1	T2	T3	T4	T5
β-carotene in <i>D. salina</i> (%)	0.5	0.5	0.5	0.5	0.5	0.5
β-carotene in Feed (%)	0	0.25	0.5	1.0	2.0	7.0
β-carotene in carcass (%)	0	0.00 0057	0.00 0063	0.00 003	0.00 0044	0.00 0046

Proximate Composition Analysis

Results on proximate composition analysis of shrimp carcass are shown in Table 5. Dry matter, moisture, protein, lipid, ash and nitrogen free extract (NFE) contents did not vary significantly ($P > 0.01$) between control and treatments.

Table 5: Proximate composition of *F. indicus* fed *D. salina* diets

Parameters	Control M ±SD	T1 M ±SD	T2 M ±SD	T3 M ±SD	T4 M ±SD	T5 M ±SD
Dry matter (%) ^{NS}	90.1± 00	89.9± 0.1	89.7± 0.3	90.5± 0.2	89.9± 0.1	89.9± 0.4
Moisture (%) ^{NS}	9.9± 00	10.2± 0.1	10.3± 0.3	9.6± 0.2	10.2± 0.1	10.1± 0.4
Protein (%) ^{NS}	71.2± 0.4	69.2± 0.5	69.4± 0.6	68.7± 1.0	69.6± 0.3	69.8± 1.3
Lipid (%) ^{NS}	4.9± 0.6	5.9± 00	4.6± 00	5.1± 0.3	4.8± 0.1	4.9± 0.1
Ash (%) ^{NS}	11.5± 00	11.7± 0.1	12.1± 00	11.6± 0.1	11.8± 0.4	12± 0.4
NFE (%)	2.6± 0.9	3.1± 0.5	3.6± 0.3	4.1± 1.6	3.7± 0.4	3.1± 0.3

NS $p > 0.01$; M-Mean; SD- Standard Deviation

Discussion

Results of the present study show that dietary incorporation of *D. salina* brought significant effect on growth performance in white shrimp, *F. indicus* and a dose at 2% inclusion in diet exhibited high growth rate compared to control. Similar growth enhancement was also reported in tiger shrimp, *Penaeus monodon* when *D. salina* extract was added into the supplementary feed^[3,4]. Supplementation of *D. salina* was not only improved growth, but also enhanced health, immunity and disease resistant in tiger shrimp. Growth promoting effect of *D. salina* was also reported in pacific white shrimp, *Litopenaeus vannamei* when carotenoid compounds were supplemented^[2,5]. Therefore, it is suggested that the enhanced growth observed in *F. indicus* is due to incorporation of *D. salina* in the feed. Studies show that the dose of *D. salina* required for growth enhancement in tiger prawn and pacific white shrimp are varied^[3,4]. These finding clearly reveal that the growth promoting effect of *D. salina* is dose dependent and species specific.

Several reports suggest that crustaceans have the metabolic activity to introduce structural modifications into carotenoids that they obtain from their diet, in particular, by introducing hydroxy groups at C(3) and C(3') and keto groups at C(4) and C(4'). Thus, canthaxanthin (β, β-carotene-4,4'-dione), zeaxanthin (β, β-carotene-3,3'-diol) and even β-carotene can undergo metabolic conversion into astaxanthin^[16,17]. Therefore, it can be suggested that growth promoting effect of *D. salina* found on *F. indicus* may be due to the presence of β-carotene content in it.

Water quality parameters recorded during the study was found to be within the suitable range required for the growth of white shrimp^[18]. The declining trend of water temperature during culture period was due to the start of winter season. Low concentrations of nitrate observed during culture period suggest the oxidation of ammonia^[19]. According to Avnimelech, et al^[20], only about 25% of the feed nutrients are converted into harvestable products hence contributing to high nitrogen residues in tank water, especially total ammonia nitrogen (TAN), which is the sum of both ammonia and ammonium which will adversely affect shrimp growth. This may be a reason for the elevated ammonia level recorded during later phase of culture in present study.

Result of the feed utilization study shows that incorporation of *D. salina* in diets did not affect feed consumption and assimilation. However, protein digestibility was found to be increased slightly in fast growing shrimp and this may be due to the better digestion of food. Proximate composition analysis of meat shows that there is no significant difference in shrimp meat quality which indicates that incorporation of *D. salina* did not influence meat quality of white prawn. Similar studies have also been reported in shrimp when feed additives are supplemented through diets^[21,22].

In shrimp culture industries, color is one of the major factors, which determines the market price of black tiger shrimp (*Penaeus monodon*) in the international market^[23]. Supplementation of carotenoid pigments into diets has been demonstrated to yield shrimp colour and higher pigments in shrimp^[15-17]. Moreover, carotenoid pigments have positive effect on the immunological and stress response^[24]. Several studies have been made to find alternative sources of astaxanthin and other carotenoids

such as yeast, *Phaffia sp.* and many species of algae^[25,26]. In the present study also *D. salina* diet fed shrimp showed better color than that of the control. Therefore, it is strongly opined that *D. salina* has the ability to improve shrimp colour in white shrimp, *F. indicus*.

It is considered that penaeid shrimp cannot biosynthesize carotenoids from mevalonic acid, but can alter dietary carotenoids by oxidation and deposit them in their tissues Tanaka, et al^[27] reported on the metabolism of carotenoids in kuruma shrimp and suggested that some of the dietary carotenoid pigments such as astaxanthin, β -carotene, isocryptoxanthin, echinenone, canthaxanthin, phoenicoxanthin, zeaxanthin and 4-ketozeaxanthin were converted into astaxanthin in shrimp body. Especially, astaxanthin was the most effective substance for pigmentation in shrimp when compared with β -carotene and canthaxanthin^[28]. However, Chien et al^[17] studied the pigmentation of the black tiger shrimp by feeding the shrimp with diets containing different carotenoid sources, e.g., β -carotene, *Spirulina*, *Phaffia* yeast and krill oil. A marked increase of carotenoid content in the carapace was observed in the group fed with *Spirulina* supplemented diets and suggested that zeaxanthin, which is the major carotenoid in *Spirulina*, was rapidly converted to astaxanthin. Moreover, 125 mg/kg of synthetic β -carotene or 125–175 mg/kg β -carotene extracted from *D. salina* have been reported as a pigment sources in black tiger shrimp and demonstrated that black tiger shrimp has the metabolic ability to converted β -carotene into astaxanthin^[3]. Based on above observations, it is suggested that *F. indicus* has the ability to convert the carotenoid content in *D. salina* and this may be a reason for the better colour development in *F. indicus*.

From previous reports, dietary carotenoids were converted into astaxanthin and deposited in the shrimp body in free form by the association with protein and exists as carotenoprotein and esterified forms which are predominantly a mono ester and di ester of long-chain fatty acids^[29]. However, the incorporation of astaxanthin esters in test diets seemed less effective than the free form as reported by^[17]. The results of the present study shows that *D. salina* incorporated diet fed shrimp had better colour than that of the control shrimp. Hence it is suggested that the carotene content in *D. salina* plays an important role on pigmentation in white shrimp as in the case of other shrimps.

Result of the present study show that shrimp body composition was not influenced by *D. salina* incorporation. The light sweet taste of treatment shrimp can be correlated to the high saline conditions of the Red Sea water used for culture. HPLC analysis on residual β -Carotene content in meat of experimental shrimp shows that presence of β -carotene content at very low level and it did not show significant difference from that of the control. The enhanced color noticed in the shrimp fed *D. salina* did not change during cooking and it is suggested that the developed color will not change while cooking. In conclusion, it is suggested that carotene content in *D. salina* play a significant role in enhancing growth and coloration in white shrimp, *F. indicus*.

Methods

The study was conducted for a period of 180 days in fiber glass tanks (500L) at the University Fish Farm at Obhur, Jeddah. There were control and treatment for the study and both

were laid out in a completely randomized design. Healthy and uniform size juvenile (4.4 ± 0.1 ; 9.2 ± 0.1 cm) produced at the Farm Hatchery were stocked at the rate of 20 pieces/tank. Dry powder of *D. salina* was procured from National Aquaculture Group (Naqua), Jeddah, Saudi Arabia and incorporated at 0.5, 1.0, 2.0, 4.0 and 8.0% in the diet and designated as treatments T1, T2, T3, T4 and T5 respectively. *D. salina* free diet was considered as control. A standard fishmeal based pellet feed (Naqua, Jeddah) having 35% protein in diet was selected for *D. salina* incorporation and the details of feed ingredients are shown in Table 6. Required quantity of *D. salina* (powder) was thoroughly mixed with soaked feed and extruded out through a pelletizer having 2m in the die. The pellets were then oven dried at 60°C and kept it in airtight containers for storage. Feeding was done at 5% of the biomass daily at 6:00 AM, 1 PM and 6 PM. Water exchange was done at the rate of 20% every day. Shrimp were sampled fortnightly to record their total length and weight and the feed quantity was re adjusted after every sampling. Specific growth rate was calculated as $\text{Loge } W_2 - \text{Loge } W_1 / T_2 - T_1$ (where W_2 is the weight of shrimp at time T_2 and W_1 is the weight of shrimp at time T_1). Water quality parameters such as temperature, dissolved oxygen, pH and salinity (HAAC, HQ40d multiply portable device meter) were recorded daily. Ammonia (unionized), nitrates (NO_3) and nitrites (NO_2) were recorded (HAAC, device colorophotometer – DR 3900) weekly. On 180th day, the experiment was terminated and total biomass and survival was calculated.

Table 6: Feed ingredients in the supplementary feed

Ingredients	Incorporation (%)
Fish meal	18.00
Soya meal	40.00
Wheat	31.78
Fish oil	3.50
Sea weed powder	0.50
Mono sodium phosphate	0.38
Mono calcium phosphate	0.50
Yeast autolyzate	0.50
Soya lecithin oil	2.53
Anti-oxidant	0.10
Anti-mould	0.15
Binder	1.00
Vitamin premix	0.35
Mineral premix	0.25
DL Methionine	0.27
Vitamin C	0.14
Lysine (Amino acid)	0.05
Total (g)	100

A short term laboratory experiment was conducted in fiber glass tanks (50 liter) using 10 shrimps (10.0 ± 1.2 g) for 30 days to assess the feed intake, conversion efficiency, and apparent protein digestibility of the control and treatment shrimp. For this, shrimp were collected from the experimental tanks and provided with weighed amount of feed for 2 hours daily between 7.00 am and 7:00 pm. The unconsumed feed and fecal matter were siphoned out separately after 1 hour of feeding. The pooled,

dried and weighed faecal matter was used for nutrient analysis. Feed conversion efficiency and nutrient digestibility were calculated as: Feed conversion efficiency (FCE %) = Wet weight gain (g) / Feed consumed (g) x 100; Apparent protein digestibility (%) = Protein in feed - Protein in excreta / Protein in feed x100.

A panel of experts evaluated the physical quality of shrimp such as colour, loose shell, soft shell, hard shell and taste after the experiment. Proximate analysis of shrimp carcass was analyzed for the estimation of protein, lipid, glycogen, fiber and ash[30]. Experimental feed, shrimp samples and dry powder of *D. salina* were subjected for the estimation of β -carotene content by High Pressure Liquid Chromatography (HPLC)^[31] and the samples were analyzed at IDAC lab, Riyadh, Saudi Arabia. One way analysis of variance (ANOVA) was employed to find out the statistical difference in growth, water quality parameters and physical quality of shrimp between control and treatment^[32].

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