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USING *DUNALIELLA SALINA* EXTRACT TO IMPROVE SURVIVAL, STRESS TOLERANCE AND GROWTH PERFORMANCE OF FRESH WATER PRAWN *Macrobrachium rosenbergii.*

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SUMMERY

A study was conducted to determine the effects of commercially available *Dunaliella salina* extract (DSE) on growth, survival and stress resistance of fresh water prawn (*Macrobrachium rosenbergii*). Thirty postlarvae (PL28) with an average individual weight of 0.032± 0.002g were stocked in circular fiberglass tanks (each 1.5 m^3 with capacity of 1000 l) for a period of 8 weeks. Five levels of DSE (200, 400, 600, 800 and 1000 mg DSE /kg) were tested. A low dissolved oxygen stress test was conducted 10 days later and prawn survival time was compared among treatments. After eight weeks rearing control prawn had significantly lower survival % than DSE supplemented diets at high doses 400-1000 mg/kg). The highest final body gain was at 600 DSE mg/kg diet. When subjected prawns to low dissolved oxygen stress test, the control treatment showed the lowest survival percentage of prawn than fed DSE diets. In conclusion, the DSE showed significant effects on survival, growth performance and feed utilization of *M. rosenbergii* PL especially those received the 600 mg DSE/kg diet.

Keywords: *Dunaliella extract, Macrobrachium rosenbergii, feed supplement, survival rate, growth performance, feed utilization.*

INTRODUCTION

Despite the more optimistic projections still forecast a certain increase in Egyptian Aquaculture markets during the next years. In upcoming years, hatcheries will play more significant role as contributors to shrimp farming. Already hatcheries are easing the demands for wild postlarvae through production of domesticated stocks. Although current hatcheries practices are enable the supply of sufficient number of fry, larval quality has become a major concern. Available methods to estimate larval quality involve the exposure of the animals to short extreme environmental stress. Shrimp larval quality is a major concern for production purposes (New, 2005 and GAFRD, 2007).

Macrobrachium rosenbergii (*M. rosenbergii*), has been studied, developed and received considerable attention over the past 20 years because of its characteristics as an

aquaculture species. High larval mortality and the long larval cycle are constraints to *M. rosenbergii* commercial rearing (FAO, 1998). After hatching, the zoea seems to have reserves for the first 24h, thus food is normally not provided during day 1. However, in the wild, where a wide rang of preys is available, it is possible that young larvae show some level of feeding activity immediately after release. Newly hatched captive larvae showed peristaltic hindgut movements, indicating swallowing of water and capacity to ingest food immediately after hatching. Without food, they lost coloration, reduced swimming activity, and some died before completing one day (Tidwell *et al.*, 1993; Tidwell *et al.*, 1994). This indicates an early start of feeding activity, even before the reserves are exhausted. Thus, it was hypothesized that supplying microalge during the following days would prevent their decline, speed their own to support *M. rosenbergii* larval development. It was also hypothesized that microalgae on the first day may boost energy resources and improve the gut condition, resulting in better digestive capacity (Cavalli *et al.*, 1999 and Rozihan *et al.*, 2001).

Naturally occurring carotenoid pigmentation in microalge diets, may be applied in animals feed so that resulting food products are appealing to the consumer. Carotenoids are amongst the most widely distributed pigments in biological systems. In natural, animals utilize color to assess the attractiveness of mate for breeding, good coloration in many animals good foraging ability (Møller *et al.*, 2000). Enhanced coloration through carotenoid deposition also signifies better health, as carotenoids have antioxidant functions, and therefore individuals with less color likely to be those with a large detoxification burden. It has even been shown that animals infested with parasites are less able to deposit caroteoids. Therefore, it may be concluded that, in animals, color often equals health and mate suitability (Guerin, *et al.*, 2003).

Survival improvement in Kuruma prawn, *Marsupenaeus japonicus* (Thongrod *et al.*, 1995), by dietary astaxanthin supplementation was reported. Recent studies showed that enhancement of resistance to oxygen depletion stress (Chien *et al.*, 1999), salinity stress (Darachai *et al.*, 1998; Merchie *et al.*, 1998; Chien *et al.*, 2003) in penaeid postlarvae was associated with an increase in dietary and body Astaxanthin. It has several essential biological function including protection against oxidation of essential polyunsaturated fatty acids; protection against UV light effects; immune response; pigmentation; communication; reproductive behavior and improve reproduction (Lorenz and Cysewski 2000). Astaxanthin cannot be synthesized by animals and must be acquired from the diet. Although mammals and most fish are unable to convert other dietary carotenoids into astaxanthin, crustaceans have limited capacity to convert closely related dietary carotenoids into astaxanthin directly (Jyonaechi *et al.*, 1995). However, synthetic astaxanthin is still expensive for use in aquaculture. Several attempts have been made to find alternative source of astaxanthin and other carotenoids such as yeast, Phaffia sp. (Sanderson and Jolly, 1994), and many species of algae (Liao *et al.*, 1993; Sommer *et al.*, 1991; Boonyaratpalin *et al.*, 2000). In this study, different levels of carotenoid extract from the green alga, *Dunaliella extract* (DSE) was used in prawn feed in order to study the effect of this material, at different levels on improving, survival %, stress resistance and growth performance in fresh water prawn.

MATERIALS AND METHODS

Dunaliella salina extract (DSE):

Dunaliella extract (DSE) was provided by El-Max Salines Company in Alexandria.

Tested diet and feed processing:

This study was undertaken at Animal and Fish Department, Faculty of Agriculture (Saba Basha), Alexandria University for 56 days. Six tested diets were formulated to have the same basic ingredients and composition with different levels of DSE (Table 1). Such levels were 0, 200, 400, 600, 800 and 1000 mg DSE /kg respectively. All ingredients (Table 1) were well ground, weighed, mixed and oil was added and mixed well. After that 350 ml of freshwater/kg feed was added and mixed well for 10 min. Then, passed through the meat grinder, the spaghetti-like feed was broken into very small pellets and dried at 60°C until the moisture was less than 10%. Dried diets were passed through 0.3 mm screen.

Table (1): Feed ingredient and chemical composition (%) of the basal experimental diet used for feeding fresh water prawn (*M. rosenbergii*) post-larvae reared in fiberglass tanks.

Item	g/kg
Fish meal	250
Prawn meal	180
Soybean meal	270
Wheat flour	230
Rice flour	62
Vitamin& Mineral mix	3
Corn Oil	5
Chemical composition (%):	
Dry matter	90
On dry matter basis (%)	
Crude Protein	40.1
Crude fat	10.3
Nitrogen free extract	33.6
Crud fiber	8
Ash	8
Gross energy Kcal/100g dry matter	461.5
Protein to Energy Ratio (mg protein/Kcalenergy)	86.9

Diet containing 40 % crude protein. Vitamin and mineral mixture contains 4800 IU Vitamin A, 2400 IU cholecalciferol (Vitamin D), 4.0g Vitamin E, 8g Vitamin K, 4.0g Vitamin B12, 4.0g Vitamin B2, 6.0g Vitamin B6, 4.0g pantothenic acid, 8.0g nicotinic acid, 400mg folic acid, 20mg biotin, 200mg choline, 4g copper, 0.4g iodine, 12g iron. Gross energy (Kcal/100g DM), calculated on the basis of 5.64, 4.11 and 9.44 Kcal GE/g protein, NFE and lipid , respectively (NRC, 1993)

Tested animals:

Postlarvae of *M. rosenbergii* with an average initial body weight 0.035 ± 0.002g were used. Twelve circular fiberglass tanks (each 1.5 m^3 with capacity of 1000 l) were assigned in this experiment. Prawns were randomly stocked into all treatment at a rate of 30 prawns in each tank, with two replicate per treatment. Each fiberglass tank was provided with four black polyvinyl chloride pipe (30 m long and 16 mm diameter) to minimize the cannibalism. The experimental tanks were cleaned every day before the first feeding and about half of the water was replaced with de-chlorinated tap water. Water temperature was checked daily, and ranged between 25 to 27°C under control. Supplemented aeration was provided through air stones by using air pumps, which permitted a suitable level of dissolved oxygen for prawn. Animals were maintained on an artificial light photoperiod of 12/12h light: dark schedule. Animals from each replicate were weighed every 2 weeks and the daily amounts of food were readjusted as percentage of live body weight at feeding rate of 20% for 8 weeks.

Growth performance analysis:

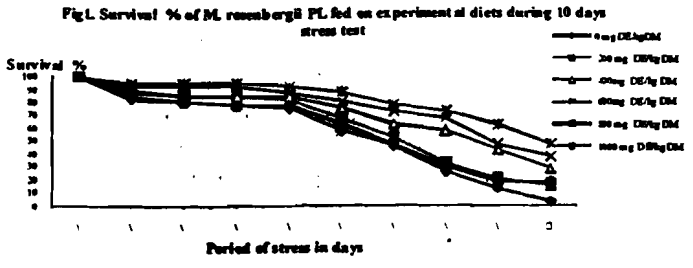
At the end of the experiment, samples of prawns were weighed and the average total weights in each treatment were calculated. The final weight, average daily gain, specific growth rate, survival %, protein efficiency ratio and food conversion ratio, Carcass composition and chemical analysis of the basic diet were estimated and performed using standard AOAC, (1990) methods.

Stress tolerance:

At the termination of the feeding experiment (8 weeks), 50% of the survived prawns from each treatment were moved in duplicates to stress test glass aquaria (30x40x100 cm^3). Prawns were stressed under low dissolved oxygen conditions by stoping the aeration and overlying plastic sheet on the water surface in each aquaria for 10h. Oxygen meter measured dissolved oxygen (YSI model 57). Oxygen level was linearly decreased to 0.5-1mg/l within the10 h (7.00 am –5.00 pm) per day and then returned to normal condition in the evening. This challenge test was run for 10 consecutive days. Mortality of the prawn in each group was recorded for 10 days period. All data were analyzed using one-way ANOVA (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Growth, survival, FCR and PER of *M. rosenbergii* fed with varying levels of DSE after 56 days of feeding are presented in Fig 2 and (Tables 2 and 3). Weight gain of prawn fed diet supplemented with 200-800 mg/kg DSE was significantly higher that of the prawn fed control diet ($P<0.05$). During the period of study, there were significant differences ($P<0.05$) in terms of body weight between diets 200, 400, 600 and 800mg DSE/kg from one side and 0 and 1000mg DSE/kg on the other side (Table 2). Diet contained 600mg DSE/kg showed the highest mean final body weight (0.744 g) followed by diets contained 800, 200, 400,0 and 1000mg DSE/kg with values 0.565, 0.532, 0.448, 0.294 and 0.257g respectively. The specific growth rate in diet contained 600mg DSE/kg was the highest and showed significant difference ($P<0.05$) compared to diets contained 0 and 1000mg DSE/kg (Table 2). However, it was not significantly different in FCR and PER among all treatments ($P>0.05$). During the10 days of stress period, the survival of fresh water prawn fed diet containing 800mg/kg DSE was the highest as shown in Fig 1 and Table 4. Moreover, survival percentages of all groups, fed DSE supplemented diets were significantly higher than the control ($P<0.05$).



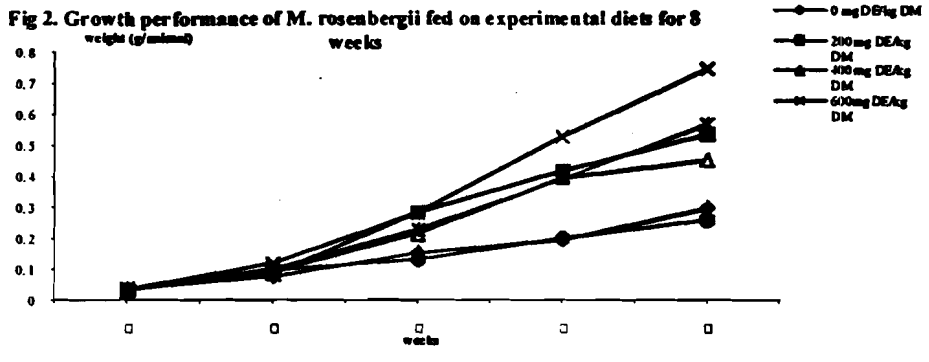


Table (2): Growth performance and feed utilization of freshwater prawn *M. rosenbergii* post larvae fed with different dietary levels of DSE.

DSE levels Mg/kg D.M	Initial Weight (g)	Final Weight (g)	Average Daily gain (g)	Survival (%)	SGR (%)	FCR	PER
0 mg/kg	0.034	0.294 ^{bc} (± 0.012)	0.005	50.56^{b}	0.464^{b} (±0.160)	3.01^{a} (± 0.321)	0.971^{a} (± 0.053)
200 mg/kg	0.030	0.532^{ab} (± 0.015)	0.009	48.89^{b}	0.896^{a} (± 0.413)	2.46^{a} (± 1.310)	1.020^{a} (± 0.256)
400 mg/kg	0.034	0.448^{ab} (± 0.043)	0.008	50.67^{b}	0.739^{a} (± 0.179)	2.59^{a} (±1.162)	1.187^{a} (± 0.273)
600 mg/kg	0.033	0.744^{a} (± 0.021)	0.013	61.11^{a}	1.270^{a} (± 0.403)	2.16^{a} (± 2.121)	1.140^{a} (± 0.161)
800 mg/kg	0.35	0.565^{ab}± 0.004	0.010	60.67^{a}	0.946^{a} ± 0.104	2.39^{a} ± 0.670	1.000^{a}± 0.025
1000mg/kg	0.032	0.257^{bc} ± 0.024	0.004	70.00^{a}	0.400^{b} ± 0.228	3.07^{a} ± 0.061	0.997^{a} ± 0.092

Mean in the same column sharing the same subscript are not significantly different (P≤0.05), SGR : Specific growth rate (%), FCR: Feed conversion ratio, PER: Protein efficiency ratio.

Table (3): Average of growth performance of *M. rosenbergii* fed on experimental diets containing different dietary levels of DSE (mg/kg DM) for 8 weeks.

Item	No. of weeks				
	0	2	4	6	8
0	0.034	0.075	0.15	0.1925	0.2945
200	0.03	0.085	0.2815	0.415	0.5325
400	0.034	0.09	0.212	0.3935	0.4485
600	0.033	0.1175	0.282	0.5265	0.744
800	0.035	0.1025	0.2265	0.3915	0.565
1000	0.032	0.097	0.1305	0.198	0.257

Table (4): Effect of different levels of dietary DSE on the average of survival (%) of *M. rosenbergii* in each treatment during 10 days of stress test period.

Item	No. Of theDays of the stress test									
	1	2	3	4	5	6	7	8	9	10
0	100	82.5	80	77.5	75	57.5	45	25	12.5	2.5
200 mg/kg	100	87.5	85	85	82.5	67.5	52.5	32.5	20	15
400mg/kg	100	90	85	85	85	75	62.5	57.5	42.5	27.5
600mg/kg	100	92.5	92.5	92.5	87.5	80	72.5	67.5	47.5	37.5
800 mg/kg	100	95	95	95	92.5	87.5	77.5	72.5	62.5	47.5
1000 mg/kg	100	85	80	77.5	77.5	62.5	47.5	30	17.5	17.5

Effects of DSE on carcass composition of *M. rosenbergii* are presented in Table 5. The obtained results revealed significant ($P<0.05$) positive effect of DSE supplements ($P<0.05$) at high doses of DSE (400, 600, 800 and 1000 g/kg DSE) on all carcass composition parameters. There was a significant ($P<0.05$) difference in dry matter content between animals fed diets with high DSE content (400, 600, 800 and 1000mg/kg) than the other treatments (control and 200mg/kg). There was a significant difference ($P<0.05$) in carcass crude protein between the control group and animals fed on DSE supplemented diets. Meanwhile there were no significant differences ($P>0.05$) in carcass crud protein among animals that fed on DSE supplemented diets. Carcass ether extract increased ($P<0.05$) with increasing DSE supplement in diets. Carcass ash content has been increased significantly ($P<0.05$) for animals that fed on DSE supplemented diets.

Table (5): Carcass composition of *M rosenbergii* fed different dietary levels of DSE (mg/kg DM).

Item	% on DM basis					
	DM	CP	EE	CF	NFE	ASH
0	25.365^{ab} (±0.190)	54.762^{a} (±0.620)	8.650^{a} (0)	3.957^{a} (±0.102)	14.785^{cd} (±0.558)	17.860^{a} (±0.014)
200	26.150^{a} (±0.707)	57.640^{b} (±0.296)	8.700^{b} (±0.070)	3.765^{a} (±0.255)	12.915^{cd} (±0.004)	16.985^{a} (0.063)
400	26.805^{bc} (±0.247)	58.310^{b} (±0.466)	10.465^{c} (±0.063)	4.600^{b} (±0.127)	11.265^{ab} (±0.318)	15.360^{b} (±0.141)
600	26.647^{bc} (±0.166)	57.880^{b} (±0.339)	10.690^{c} (±0.353)	4.565^{b} (±0.063)	11.455^{ab} (±0.318)	15.410^{c} (±0.070)
800	26.655^{bc} (±0.233)	59.115^{b} (±0.813)	10.620^{c} (±0.056)	4.610^{b} (±0.162)	9.770^{bcd} (±0.014)	15.885^{c} (±0.091)
1000	26.795^{bc} (±0.120)	58.120^{b} (±0.947)	10.610^{c} (±0.070)	4.620^{b} (±0.042)	11.260^{ab} (±0.749)	15.390^{c} (±0.070)

After 3 weeks of feeding with the test diet, it was easy by naked eye to distinguish among treatments using body color. Prawn in treatments 200-1000mg DSE/kg looked dark blue, while pale blue was observed in the control treatment. The color was more intense in diet 600mg DSE/kg than the others.

Although mammals and most fish are unable to convert dietary carotenoids into astaxanthin, crustaceans (such as prawn and some fish species including carp) have a limited capacity to convert closely related dietary carotenoids into astaxanthin. Tanaka *et al.* (1976) reported on the metabolism of carotenoids in Kuruma shrimp and suggested that some of dietary carotenoid pigments such as astaxanthin, canthaxanthin, phoenicoxanthin, zeaxanthin and 4-ketozeaxanthin were converted into astaxanthin in shrimp body. Boonyaratpalin *et al.* (2001) have been reported that 125-175mg/kg of synthetic β-carotene extracted from *Dunaliella salina* (Betatene) demonstrated that black tiger shrimp has metabolic ability to converted β-carotene into astaxanthin. Also this finding agrees with Supamattaya *et al.* (2005) results, 200-300mg/kg of β-carotene from *D. salina* (Algro Natural®) showed its high efficiency for growth, survival and pigmentation in black tiger shrimp. Such finding was correlated with our results, (Table 2) 600-800mg/kg of β-carotene from *D. salina* (El-max salin Com.) showed its high efficiency in survival %, growth and pigmentation in postlarvae of *M. rosenbergii* prawn. From previous reports, dietary β-carotene from *D. salina* were converted into astaxanthinand deposited in the shrimp body in free form by the association with protein and exists as carotenoprotein and esterified forms witch are predominantly a monoester and di-ester of long-chain fatty acids (Foss *et al.*, 1987;Yamada *et al.*, 1990 and Supamattaya *et al.*, 2005). Carotenoids are fat-soluble pigments (Fox and Vevers, 1960), thus their absorption and metabolism may be also lipid-related. This may explain high significant content of ether extract and crude protein in carcass composition of animals that fed on 400, 600, 800 and 1000mg/kg DSE (Table 5). It was observed that biosynthesis or deposition of carotenoids may be adversely affected by poor lipid nutrition (Meyers and Latscha, 1997). In crustacean, unesterified and esterified carotenoids may accumulate as lipid dispersions in the chromatophores (Fingerman, 1965), binding with chitin in the carapace (Ghidalia, 1985 and Gladis and Bjørn, 2002). Studies suggested that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the principal fatty acids esterified with the potions of astaxanthin linked to chitin in the shrimp carapace (Meyers and Latscha, 1997). Chien and Jong (1992) stated that *Penaeus japonicus*, growth benefits have been recorded of feeding astaxanthin and Nèger-Sadargues *et al.* (1993) have shown a growth rate increase of least 25% through the addition of astaxanthin to a non-pigmented basal feed. In the study of Thongrod *et al.*, (1995), however, an increase in growth was observed in *P. monodon* fed with increasing levels (5-300mg/kg) for 30 days. Petit *et al.*, (1997) also found that dietary astaxanthin improved the growth rate and shortened the moulting cycle of *M. japonicus* postlarvae during 20-day rearing period. All above- mentioned factors could have introduced complexity and variation in pigmentation and made the differences final weight, FCR, SGR. Our results stated (Fig 2) that there is some evidence showed the positive effect of dietary β-carotene from *D. salina* on growth (SGR 200-800mg/kg β-carotene from *D. salina*) but no evidence showed the positive effect on feed efficiency, FCR and PER *M. rosenbergii*. Moreover 1000mg/kg β-carotene from *D. salina* has the poorest growth, effect on feed efficiency, FCR and PER. Johnson and An (1991) suggesting that because of the thick cell wall of algae may impede the digestibility and absorption. Moreover, a high supplementation of dried algal cells caused retardation of growth in black tiger shrimp (Lio et al., 1993). In Atlantic salmon parr, Christiansen, *et al.* (1995) were able to show that fish

fed feed containing 60 mg astaxanthin per kg grew significantly better than fish a diet free of this pigment. An essentiality of astaxanthin for growth and survival in these fish was claimed, and Torrissen and Christiansen (1995) subsequently recommended that a minimum of 10mg astaxanthin or canthaxanthin per kg diet should be supplemented into feeds for all fish and crayfish diets. In Larvae and juveniles of non-salmonid fish too, they have reported slight growth benefits, for example in carp and tilapia. Senger *et al.*, (1989) reported that astaxanthin has a positive nutritional function in the intermediary metabolism and liver functioning (astaxanthin supplementation have beneficial to liver structure and also hepatic glycogen storage) in tilapia. On the other hand Kalinowski *et al.*, (2005) found that fish fed carotenoids did not improve growth performance and FCR (Chebbaki, 2001 and Gomes *et al.*, 2002). Also, a study with rainbow trout (Nickell and Bomage, 1998 and Yanar *et al.*, 2007) found no significant differences. Nevertheless, with rainbow trout, fish fed an astaxanthin supplemented diets from 6.5 and 25.5 exhibited higher wet weight than fish fed astaxanthin from120.5 up to 400 g; these results suggest supplementing for longer periods are need to evaluate a possible role on growth.

Lower survival % (Table 2) of diets1, 2 and 3 than high supplemented DSE 600, 800 and 1000mg/kg dietary β-carotene from *D. salina* was sufficient to sustain prawn survival. The similar survival % between prawns fed pigmented diets at different concentrations (600 and 800 mg/kg) indicated that carotenoids supplemented at 800 mg/kg had no extra benefit on prawn's survival compared with lower supplemented diets (200, 300 and 400 mg/kg). Yamada *et al.* (1990) also reported that *M. japonicus* fed with diets supplemented with astaxanthin (50-400mg/kg) had higher survival than those fed with a control diet. Chien and Jong (1992) also found a positive correlation between survival and optimal pigment concentration in prawn tissue and suggested that pigment might play a role in improving the survival of prawn. Bordner *et al.* (1986) pointed out that disregarding the concentration, as long as carotenoid was supplemented in the diet, the survival of crustacean would not be affected.

Low dissolved Oxygen stress test:

Control group exhibited very poor resistance to low oxygen stress conditions compared with animals fed with β-carotene from *D. salina* supplemented diets (Fig 1). Similar trend was reported by Chien *et al.*, (1999) on juvenile *P. monodon* fed with diet without astaxanthin supplement compared with those fed with 360mg/kg astaxanthin for 1 week. The possibility that oxygen- containing carotenoids such as astaxanthin and lutein (Soin, 1954; Czecuga, 1979), In which oxygen is attached at the center of the hydrocarbon chain (Karnaukhov, 1979), may act as an intracellular oxygen reserve for respiration in salmonids eggs subjected to oxygen stress has been postulated (Craik, 1985), Such intracellular oxygen reserves (Ghidalia, 1985; Latscha, 1990; Oshima *et al.*, 1993) or of its electron-acceptor equivalent (Ghidalia, 1985) may also permit the crustacean to survive under hypoxic conditions common in pond culture environments (Chien and Jong, 1992). High astaxanthin content in kuruma prawn, about 90%of its total pigments (Ishikawa *et al.*, 1966), may contribute to its high tolerance to low dissolve oxygen and lower demand for oxygen. This is evidenced by the fact that kuruma prawn is one of the few penaeids that can be transported alive without water and the fact that during the day, it burrows itself into the sediment (Bailey-Brock and Moss, 1992), which is usually hypoxic. Aside from being active in cross-membrane calcium transfer and serving as oxygen reservoirs in the neuronal respiratory chain carotenoids also protect sensitive tissues and reactive compounds from damage due to oxidation (Oshima *et al.*, 1993). There have been several reports suggesting that astaxanthin functions as a potent antioxidant for prawn against

physical (Darachai *et al.*, 1998; Merchie *et al.*, 1998; Chien *et al.*, 2003), Chemical (Pan *et al.*, 2003a), and pathological stresses (Merchie *et al.*, 1998; Pan *et al.*, 2003b). Astaxanthin may serve as an intracellular oxygen supply for shrimp, allowing survival under the hypoxic conditions in the pond bottom (Chien and Jong, 1992). Chein *et al.* (1999) reported that black tiger shrimp fed diet containing 360 mg synthetic astaxanthin/kg diet had better survival when exposed to oxygen depletion stress. This finding agreed with our study and concluded that deposited β-carotene from *DSE* extract may constitute an intracellular reserve of oxygen in black tiger shrimp.

All above results concluded that increasing DSE (from El-Max salin company) level in *M. rosenbergii* diets resulted in better survival, growth and quality of *M. rosenbergii* PL.

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استخدام مستخلص طحلب الدونناليلا سالينا لتحسين الاعاشة والمقاومة للاجهاد واداء النمو فى جمبرى المياه العذبة ماكروبراكيم روزنبرجى

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اجريت الدراسة لتقييم إضافة مستخلص طحلب الدونالیلا على كل من النمو والاعاشة ومعدل الإجهاد لجمبرى المياه العذبة ماكروبراكيم روزنبرجى.٣٠ يرقة عمر ٢٨ يوم بمعدل وزن ٠.٠٣٢ جرام تم تخزينها فى تانكات فيبرجلاس (١.٥ م^{٣} تم ملئوه بـ ١٠٠٠ لتر) لمدة ٨ اسابيع.

٥ مستويات من مستخلص الدونالیلا تم اضافتها للعليقة (٢٠٠ ، ٤٠٠ ، ٦٠٠ ، ٨٠٠ و ١٠٠٠ ملليجرام مستخلص الدونالیلا /كجم من المكونات الجافة للعليقة. تم اختبار الاجهاد بخفض نسبة الاكسجين لمدة ١٠ ايام بعد انتهاء التجربة وتم مقارنة معدلات الاعاشة نتيجة للاجهاد بخفض نسبة الاكسجين. اوضحت النتائج بعد انتهاء فترة التجربة انخفضت النسبة المئوية للاعاشة فى العليقة الخالية من مستخلص الدونالیلا عن العلائق المضاف اليها ٤٠٠ الى ١٠٠٠ ملليجرام/كجم . اعلى معدل زيادة فى الوزن كان عند مستوى اضافة ٦٠٠ ملليجرام /كجم. وكانت ايضا معاملة خالية من مستخلص الدونالیلا اعلى معدل وفيات بعد اختبار الاجهاد نتيجة لخفض الاكسجين ومن النتائج السابقة تلاحظ أن اضافة مستخلص الدونالیلا كان لها تأثير معنوى على الاعاشة والنمو واداء النمو ليرقات جمبرى المياه العذبة