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Effects of dietary κ-carrageenan on growth and resistance to acute salinity stress in the black tiger shrimp *Penaeus monodon* post larvae

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Key words: sulfated polysaccharide, *Kappaphycus alvarezii*, refined seaweed extract, immune enhancer, attractability, salinity stress

Abstract

Acute salinity stress happens unnoticed in shrimp ponds and there is a need to protect the animals by providing dietary functional ingredients that will elicit resistance to this stress. κ-Carrageenan, a sulfated polysaccharide from red seaweed, is reported to trigger innate immunity *in vitro* and *in vivo* in shrimps. The present study aims to evaluate its potential as growth-promoter and its immune-enhancement effects against salinity stress. Three separate experiments were done: (a) an attractability test; (b) a 30-day feeding trial to determine optimal inclusion of κ-carrageenan, and (3) acute salinity stress test. Results of the attractability tests showed that all the experimental diets did not differ in their attractability to the shrimps. In the feeding trial, 5 groups of post larval shrimp were fed with 5 experimental diets containing various inclusion levels of κ-carrageenan, namely, 0.0, 0.15, 0.30, 0.45, and 0.60 g kg^{-1}. Results showed that the final average body weight (FABW), weight gain (WG), and specific growth rate (SGR) and feed conversion efficiency (FCE) values of shrimps in the 0.15 and 0.3 g kg^{-1} were significantly higher than that of the control group; the values of those in 0.45 and 0.60 g kg^{-1} groups were not significantly different from those of the control group. Protein efficiency ratio (PER) values, in contrast, were not significantly different from each group. Optimal inclusion level in the diet of κ-carrageenan was 0.29 g kg^{-1}. In the acute salinity stress test, 5 groups of shrimps were fed with the same 5 experimental diets as those in the feeding experiment for 2 weeks, transferred them from 21 ppt salinity media to 4 ppt. Results showed that the control group exhibited significantly highest mortality rate while in the 0.6 g kg^{-1} group the lowest; each group were significantly different from each other. Mortality rate decreased with increased dietary κ-carrageenan level in a linear fashion. In conclusion, the study demonstrated that the the diets containing dietary κ-carrageenan was equally as attractive as the basal/control diet and that κ-carrageenan showed both growth-promoting and immune-stimulating effects against acute salinity stress in the black tiger shrimp *P. monodon* post larvae.

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Introduction

Disease outbreaks caused by bacterial pathogens continue to be a major threat in the in many shrimp-producing countries in the world. High virulence of *Vibrio* spp. to penaeid shrimps is highly affected by the fluctuations in environmental parameters (Kautsky et al., 2000). The black tiger shrimp, *Penaeus monodon*, is a euryhaline species that can tolerate a wide range of salinities from 1 ppt to 57 ppt (Chen, 1990); its iso-osmotic level is 750 mOsm kg^{-1} (equivalent to 25 ppt, Cheng & Liao, 1986) and it is often cultured between 10 and 20 ppt in which they exhibit improved growth (Fang et al., 1992). Fluctuations in salinity are almost inevitable and go unnoticed specially during rainy season; abrupt salinity reduction may cause stress to the animals. It is possible that acute salinity changes that occur over a time weakens their immune system, leading to their vulnerability against opportunistic pathogens like *Vibrio* spp. There are evidences of metabolic changes and immune depression in the white shrimp *Litopenaeus vannamei* in response to variations in salinity (Wang & Chen, 2005). There is a need to explore functional dietary ingredients that would elicit a kind of environmental adaption in the cultured shrimps.

In invertebrates, responses to environmental stress or to disease pathogens are through nonspecific defense mechanisms since they do not possess other specific defenses. Responses to stress in shrimp are improved with the addition of dietary immunostimulants (Raa, 1996; Sakai, 1999). Seaweeds are sources of immunostimulants for shrimps such as hot-water extract of *Gracillaria tenuistipitata* (Yeh & Chen, 2009), *Sargassum fusiforme* polysaccharide (Huang et al., 2006), sodium alginate (Liu et al., 2006), fucoidan (Kitikiew et al, 2013). Carrageenan, a red seaweed polysaccharide, has been demonstrated in *Litopenaeus vannamei* to increase resistance against *Vibrio alginolyticus* (Chen et al., 2014; Ye & Chen, 2008). Carrageenan is composed of galactose and 3,6-anhydrogalactose monomers with etherified sulfate connected to calcium, sodium, and potassium (Van Doan et al., 2019). Kappa type carrageenan (κ-carrageenan) has one ester sulfate moiety and a 3,6-anhydro-galactose content moiety (Necas & Bartosikova, 2013). Carrageenan is one of the Philippines' largest exports globally (Simeon, L.M., 2016) and its possible use in the shrimp feed industry could boost not only the red algae aquaculture (specifically *Kappaphycus alvarezii* in the Philippines) that produces κ-carrageenan but also its shrimp aquaculture industry.

Most of the studies in the literature involving shrimps evaluated carrageenans as stimulants of humoral and cellular immune responses but very rarely their biological nutritive value for growth and efficiency. The only available literature that involved growth trial was that of Peñaflorida and Golez (1996) who evaluated the whole seaweed *Kappaphycus alvarezii*, and not carrageenan per se, as binder in the diet of juvenile *P. monodon*. Their results showed that 5% inclusion of the seaweed resulted in higher specific growth rate. The present study aimed to evaluate κ-carrageenan as to whether it had either growth-promoting or immune-stimulating effects against acute salinity stress at the organismic level in *Penaeus monodon* post larvae.

Materials and Methods

Experimental shrimps and set-up

The study was conducted in the wet laboratory of the National Institute of Molecular Biology and Biotechnology (NIMBB), University of the Philippines Visayas (UPV), Miagao, Iloilo, Philippines.

A total of 450 individuals of post-larvae black tiger shrimps *Penaeus monodon* (PL. 20; initial body weight (IBW) of 0.01 g, were randomly stocked in 15 experimental units. These post-larvae were purchased from the SNB hatchery located at Brgy. Bongol San Vicente, Guimbal, Iloilo, about 12 km to the wet laboratory of the university. The shrimp larvae which were certified White Spot Syndrome Virus (WSSV) and *Vibrio parahemolyticus*-free, were transported in plastic bags half-filled with oxygenated sea water. The shrimps were slowly acclimatized to ambient temperature and salinity in a 250-L capacity circular fiberglass tank and later to the basal (i.e. control) diet for 3 days prior to stocking into the 15 experimental tanks.

Diet preparation

Five diets containing various concentrations of refined κ-carrageenan extracted from *Kappaphycus alvarezii* (Shemberg Mktg Corp., Cebu, Philippines) were prepared, namely: 0, 0.15, 0.30, and 0.60g kg^{-1}. The diet ingredients and proximate composition are presented in **Table 1**. Dry ingredients were sieved, weighed, and thoroughly mixed in a large plastic bag after which the liquid ingredients such as lecithin, fish oil and water-dissolved refined κ-carrageenan were added. Gelatinized high-grade flour (i.e., cooked to clear consistency) was added last and manually kneaded inside the plastic bag into dough. The dough was steam for 15 min, rolled out thinly onto

a metal tray in which small square size portions were marked using a knife prior to oven drying. Oven drying was done for 24 h at 60°C or until the moisture reached <10 %. The dried experimental diets were broken to pieces, put in plastic bags, and stored at -20^{0}C until use.

Diet attractability test

Six separate attractability tests were conducted using rectangular glass tanks with multiple chambers (Suresh et al., 2011) (**Figure 1**). Each tank consisted of 3 major chambers (acclimatization chamber, middle chamber, and feeding chamber) that were separated with glass partitions. The feeding chamber consisted of 5 sub-chambers measuring 6 x 5 cm.

Each glass tank (90 x 50 x 30 cm in length, width and height, respectively) consisted of an acclimatization chamber at one end and feeding chambers at the other end separated by a liftable glass partition. The tanks were set up in a room with only fluorescent light. All assessments were conducted at the same time of the day commencing at 09:30 h. Diet attractability was performed on the 5 experimental diets in three simultaneous runs. Ten randomly selected shrimps were acclimatized for 1 h after which 2 g of each experimental diet were placed separately in each of the feeding chambers. Following the lifting of the glass partition, the number of shrimps that entered each of the feeding chambers was recorded. Diet attractability was expressed as percent of shrimp that were inside a particular feeding chamber containing a particular experimental diet after 1, 2, 5, 10 and 15 min following the lifting of the glass partition.

Table 1 Feed composition and proximate analysis of experimental diets containing refined κ-carrageenan for *Penaeus monodon* post larvae growth trial (g 1000 g diet^{-1}) for 30 days.

Ingredients	*Control (0.00)*	*Diet 1 (0.15)*	*Diet 2 (0.30)*	*Diet 3 (0.45)*	*Diet 4 (0.60)*
Peruvian fish meal	200.0	200.0	200.0	200.0	200.0
Shrimp meal	340.0	340.0	340.0	340.0	340.0
Soybean meal	210.0	210.0	210.0	210.0	210.0
CMC	34.8	34.65	34.5	34.35	34.2
Vitamin mix[1]	10.0	10.0	10.0	10.0	10.0
Mineral mix[2]	10.0	10.0	10.0	10.0	10.0
BHT	0.2	0.2	0.2	0.2	0.2
Lecithin	5.0	5.0	5.0	5.0	5.0
Cod liver oil	40.0	40.0	40.0	40.0	40.0
Starch	150.0	150.0	150.0	150.0	150.0
κ-carrageenan	0.0	0.15	0.30	0.45	0.60
Total	1000.0	1000.0	1000.0	1000.0	1000.0
				Proximate analysis (%, dry weight basis)	
Crude Protein	52.66	52.78	52.46	52.49	52.90
Crude Fat	7.17	7.17	7.14	7.12	7.26
Crude Fiber	2.25	2.34	2.40	2.57	2.35
Moisture	6.34	6.80	5.79	7.25	5.52
Ash	14.18	14.80	14.50	14.41	12.99
NFE	17.41	16.11	17.71	16.16	18.98

[1]Vitamin mix (mg kg^{-1} dry diet unless otherwise stated): Vitamin A 1 200,000 IU, Vitamin D3 200,000 IU, Vitamin E 20,000 IU, Vitamin B1 8,000 IU, Vitamin B2 8,000 IU, Vitamin B6 5,000 IU, Vitamin B12 2000 µg, Niacin 40,000 µg, Calcium pantothenate 20,000 µg, Biotin 40 µg, Folic acid 1,800 µg, Ethoxyquin 500 µg, Carrier q.s ad to make 1 kg; [2]Mineral mix (mg kg^{-1} dry diet unless otherwise stated): Iron 400 mg; Manganese 100 mg; Zinc 400 mg; Copper 40 mg; Iodine 18 mg; Cobalt 0.2 mg; Selenium 2 mg.

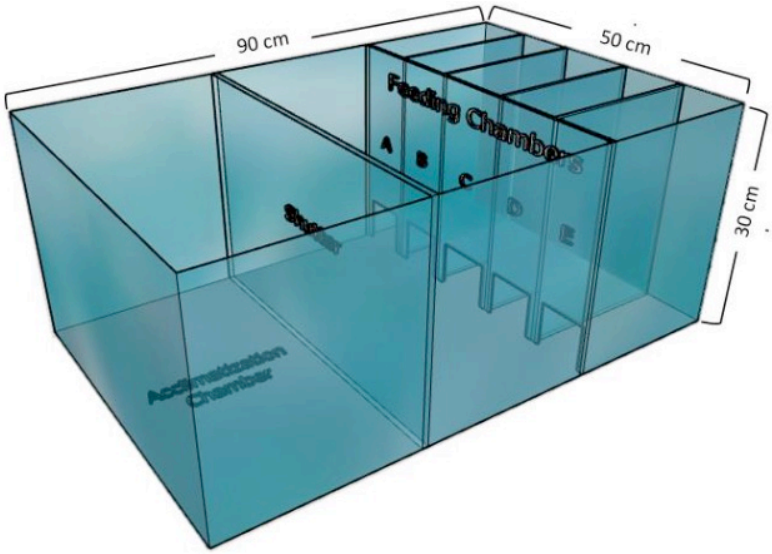

Figure 1 Schematic diagram of the tank used for the attractability test.

Feeding trial experiment
Uniformed-sized post larval shrimps were distributed into 15 experimental containers in a completely randomized design (CRD) in a recirculating system. The system comprised a reservoir into which collected effluent water from each of the experimental units was passed through a cloth fiber filter into the reservoir. Water from the reservoir was pumped onto an elevated filter (about 7 ft), and with a 50-L capacity plastic container filled with sand and gravel; the water drained onto a lower biological filter container which contained sterilized empty oyster shells. The water coming from the biological filter was distributed through a pipe system into each of the experimental units. Feeding was done five times daily at 0700, 0930, 1200, 1430 , and 1700 h. Siphoning off of about 50% of the total recirculating water volume of waste and uneaten feeds was done every morning before the first feeding and water was replaced daily. Feeding rate was at 30% body weight for the first 2 weeks and 20% for the last 2 weeks. Water quality parameters such as salinity, temperature, pH, and DO were measured daily while ammonia (NH_3/NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) were measured weekly using commercially available kits (API® MARINE).

Growth response parameters
Growth performance and feed utilization indices such as weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER), and survival rate were estimated using the following formulae:

$$WG\ (g) = IBW - FBW$$

$$SGR\ (\%\ daily) = (In\ FBW - In\ IBW) / D \times 100$$

Where: FBW - final body weight, IBW- initial body weight, D - number of days of culture.

$$FCE = wet\ weight\ gain\ (g) / feed\ consume\ (g)$$

$$PER = weight\ gain\ (g)/ protein\ fed\ (g)$$

$$Survival\ rate = (final\ number\ of\ shrimp / initial\ number\ of\ shrimp) \times 100$$

Shrimps were weighed by batch at the start and termination of the feeding experiment using a digital top-loading balance. The shrimps were carefully captured using a fine-meshed scoop net and were placed into a small clear plastic cup without water and quickly weighed.

Acute salinity stress test
A new batch of 450 of post larval shrimps were randomly distributed into a new set of 50-L containers- a total of 15 units of 50L-capacity plastic containers in a static water system. Shrimps

were fed with the experimental diets at 25% body weight for 15 days. The experimental procedure was similar to that of the growth trial except that the feeding period was only for 15 days. On the 15th day, shrimps were transferred from a 20-ppt water medium to 4 ppt in fifteen 10-L capacity plastic container and were monitored for 4 days (96h). Mortality was monitored every 15 min for 1 h, then every hour for 4 h, followed by every 4 h for 12 h. Water parameters such as pH, salinity, DO and temperature were monitored, and about 25% of water was carefully replaced daily. Shrimps continued to be fed 3 times a day with their corresponding experimental diets at 6% of ABW with proper aeration.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, version 23). Data were analyzed for homogeneity and normality of distribution and upon passing the tests, one-way analysis of variance (one-way ANOVA) was applied to test the statistical significance. Duncan's Multiple Range Test (DMRT) was used to rank the significance among treatments. The level of significance was set at α=0.05. The data was presented as mean ± standard error of the mean (SEM).

Results

The mean water quality parameters during the duration of the experiment are presented in **Table 2**. No major fluctuations occurred in the daily salinity, temperature, pH, DO, ammonia-N, nitrite and nitrate.

Results of the attractability test of the experimental diets showed no significant differences between diets indicating that diets supplemented with κ-carrageenan was at least as attractive as the control diet (**Table 3**).

Table 2 Means of water quality parameters during feeding trial of the black tiger shrimp *P. monodon* post larvae fed with various dietary kappa-carrageenan inclusions for 30 days

Water Parameters	*Treatments (g kg^{-1})*				
	Control (0.00)	*Diet 1 (0.15)*	*Diet 2 (0.30)*	*Diet 3 (0.45)*	*Diet 4 (0.60)*
Salinity (‰)	21.6±0.3	21.4±0.1	21.6±0.2	21.6±0.2	21.5±0.2
Temp. (℃)	29.0±0.5	29.3±0.4	28.8±0.4	28.8±0.4	28.9±0.4
pH	8.5±0.1	8.5±0.1	8.6±0.0	8.6±0.0	8.5±0.0
DO (ppm)	6.5±0.7	6.5±0.5	6.3±0.6	6.5±0.5	6.5±0.6
NH_3/NH_4^+ (ppm)	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
NO_2^- (ppm)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
NO_3^-(ppm)	15.0±5.5	14.2±6.7	15.0±5.5	18.3±4.1	18.3±4.1

Table 3 Attractability of shrimp *P. monodon* to the control and κ-carrageenan diets

Treatments (g kg^{-1})	*Time*				
	1 min	*2 mins*	*5 mins*	*10 mins*	*15 mins*
0.0	6.7±5.5^a	8.9±6.5^a	11.1 ±6.5^a	16.7±6.2^a	27.8±6.4^a
0.15	7.8±3.6^a	11.1±4.2^a	17.8±5.7^a	33.3±8.3^a	47.8±13.0^a
0.30	3.3±1.7^a	5.6±2.9^a	12.2±6.0^a	22.2±7.0^a	32.2±9.5^a
0.45	10.0±4.4^a	12.2±4.0^a	17.8±6.2^a	28.9±8.1^a	37.8±11.5^a
0.60	4.4±3.4^a	7.8±6.6^a	14.4±10.9^a	20.0±10.7^a	25.6±10.8^a

Sampling was done at the onset and at the termination of the trial on the 30th day of feeding with the experimental diet. The length of time of the growth trial, 30 days, was in accordance with the recommendation of Lazo et al. (2000) that in general, feeding trials involving larvae require 14-28 days, juveniles 6-8 weeks and larger fish 14-18 weeks. Results of the feeding trial in terms of growth, feed efficiency and survival are shown in **Table 4**. The overall survival rates of the experimental shrimps in the feeding trial (i.e. the first experiment) were not significantly different from each other ranging from 65.6% to 86.7%. Results of the growth trial showed that the final

average body weight (FABW), weight gain (WG) and specific growth rate (SGR) of shrimps fed 0.15 to 0.30 g kg^{-1} were significantly higher ($p < 0.05$) than those fed the control and 0.60 g kg^{-1} κ-carrageenan groups; the latter two groups were not significantly different ($p > 0.05$) from each other. For the feed efficiency parameters, food conversion efficiency (FCE) values were significantly higher in 0.15 and 0.30 g kg^{-1} groups than those in the control and 0.60 g kg^{-1} groups ($p < 0.05$); the 0.45 g kg^{-1} group was not significantly different from either of these two group pairs ($p>0.05$). Protein efficiency ratio (PER) values did not differ significantly differ in all dietary treatments.

Optimal inclusion rate of κ-carrageenan was estimated by fitting either the WG and SGR data into a second level polynomial regression, to be at 0.29 g kg^{-1} (**Figure 2**).

Results of the acute salinity stress test showed that shrimps fed 0.60 g kg^{-1} κ-carrageenan exhibited significantly the lowest mortality rate of 5.6% after 96 h following transfer from 21 ppt to 4 ppt salinity (**Figure 3**, top figure). The control group exhibited significantly the highest mortality rate of 44.4% followed by the 0.15, 0.30 and 0.45 g kg^{-1} groups in this order. Mortality rate in each dietary group was significantly different from each other and that percent mortality decreased linearly (r=0.88) with increase in dietary κ-carrageenan level (**Figure 3**, bottom figure).

Table 4 Growth performance of the black tiger shrimp *Penaeus monodon* fed with diets containing different concentrations of dietary κ-carrageenan for 30 days.

	Treatments (g kg^{-1})				
	Control (0.0)	*Diet 1 (0.15)*	*Diet 2 (0.30)*	*Diet 3 (0.45)*	*Diet 4 (0.60)*
IABW	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
FABW	$0.04±0.00^{b}$	$0.06±0.01^{a}$	$0.06±0.01^{a}$	$0.05±0.01^{ab}$	$0.04±0.00^{b}$
FI	$0.21±0.05^{b}$	$0.24±0.03^{b}$	$0.30±0.01^{a}$	$0.26±0.01^{ab}$	$0.21±0.03^{b}$
WG	$0.03±0.00^{b}$	$0.05±0.01^{a}$	$0.05±0.01^{a}$	$0.04±0.01^{ab}$	$0.03±0.01^{b}$
SGR	$3.31±0.19^{b}$	$5.05±0.90^{a}$	$5.29±0.94^{a}$	$4.00±0.80^{ab}$	$3.28±0.67^{b}$
SURV	$78.9±9.7^{a}$	$65.6±2.9^{a}$	$78.9±6.2^{a}$	$86.7±8.4^{a}$	$82.22±11.1^{a}$
PER	$0.31±0.06^{a}$	$0.40±0.04^{a}$	$0.34±0.06^{a}$	$0.29±0.07^{a}$	$0.30±0.10^{a}$
FCE	$0.53±0.03^{b}$	$0.81±0.14^{a}$	$0.85±0.15^{a}$	$0.64±0.13^{ab}$	$0.53±0.11^{b}$

Values in the same column with different superscript letters are significantly different ($p<0.05$). Values were expressed as mean ± SEM, IABW - initial average body weight; FABW - final average body weight; FI - feed intake; WG - weight gain; SGR - specific growth rate; survival - percentage survival; FCR - feed conversion ratio; PER - protein efficiency ratio; FCE - feed conversion efficiency.

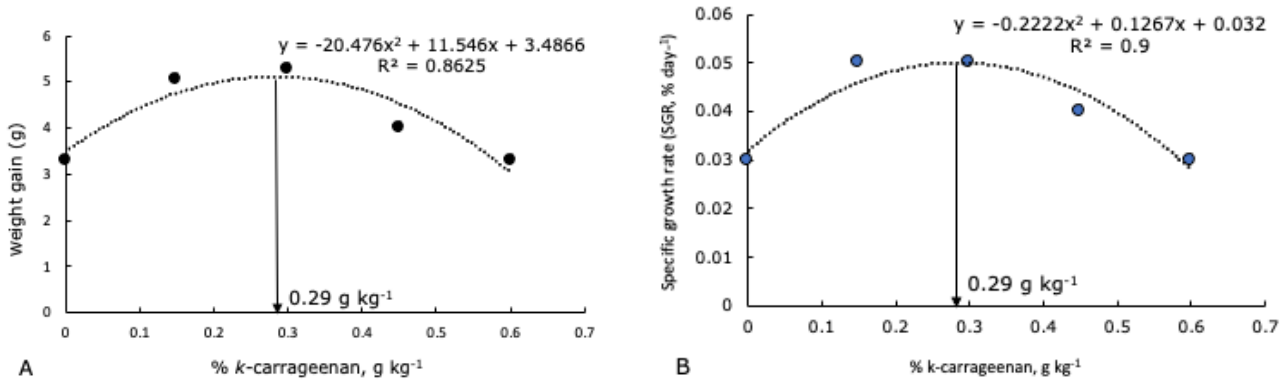

Figure 2 Optimal level of κ-carrageenan determined by fitting weight gain (A) and specific growth rate (B) data into a quadratic model.

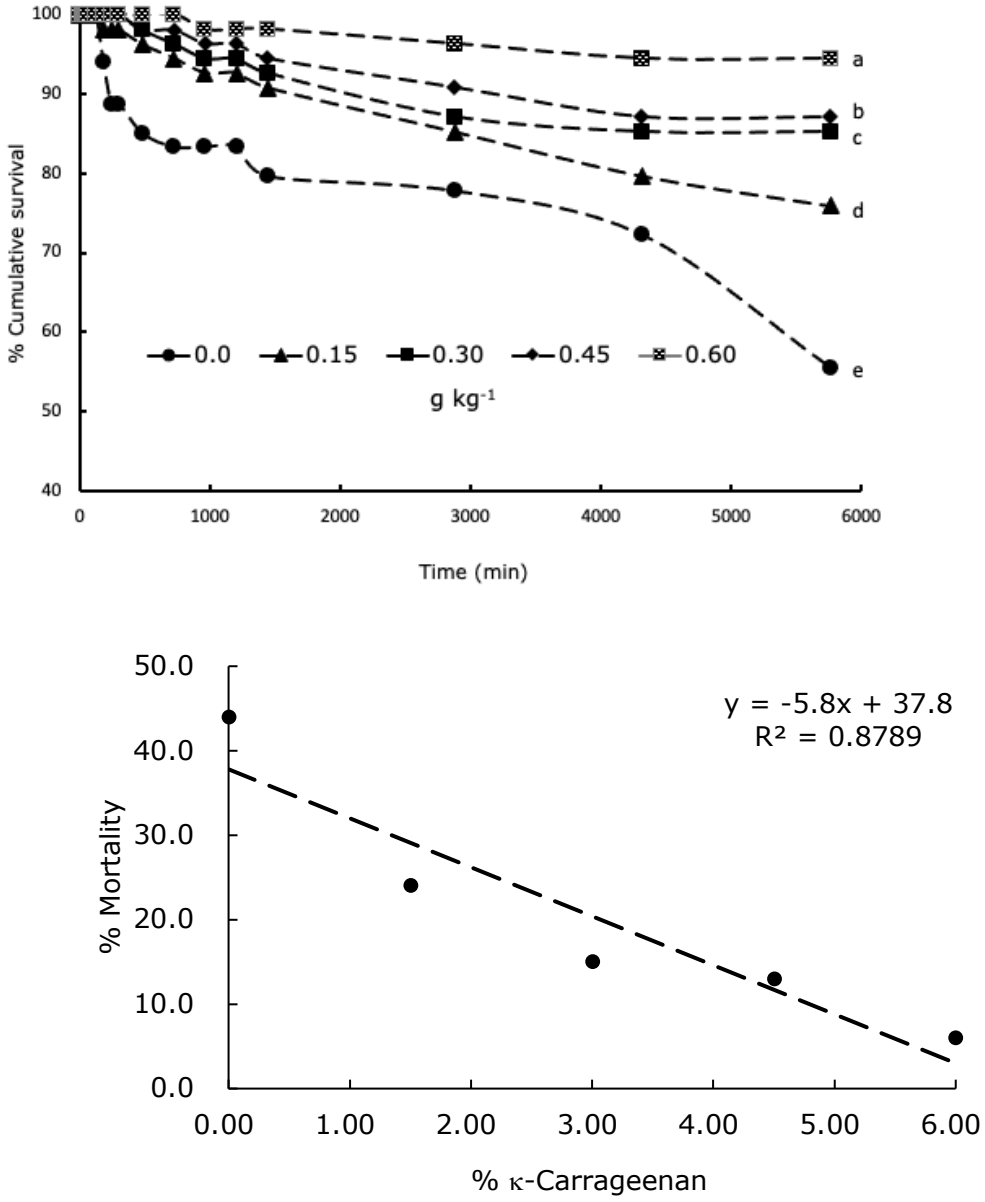

Figure 3 Cumulative mortality of *P. monodon* post larvae in the acute salinity change challenge test following feeding various κ-carrageenan inclusions for 15 days (top graph). Linear regression of the % dietary κ-carrageenan inclusion vs % mortality (bottom graph).

Discussion

κ-Carrageenan did not affect the attractability of the diet to *P. monodon* post larvae presumably because it does not have any strong aroma or flavor which is the reason why it is widely used in the food industry for its gelling, thickening, and stabilizing properties.

The effects of refined dietary κ-carrageenans on growth performance in shrimp aquaculture have been reported only by Traifalgar et al., (2013); however, a range-finding experiment has not been done until the present study. Most reports on incorporating marine macroalgae into the aquafeeds are practical in nature i.e. incorporation of the whole seaweed in the diets of shrimps and evaluating its effect on growth and feed efficiency of shrimps. Very few studies have been done on marine extracts such as carageenans or fucoidan as to their effects on growth performance in shrimps. A few have been done by our investigators such as the evaluation of ulvan, a commercial extract from *Ulva intestinalis* (formerly *Enteromorpha intestinalis*) on its effect on the growth performance of *Penaeus monodon* (Serrano & Declarador, 2014) and of fucoidan on

Penaeus monodon (Traifalgar et al., 2013). These refined extracts were also evaluated for their immunostimulatory properties on *Penaeus monodon* by our investigators (Declarador et al., 2014; Lauzon & Serrano, 2015). The present study is a range-finding study to determine the biological nutritive value of various dietary levels of refined κ-carrageenan on *P. monodon* post larvae.

It is apparent in the present study that dietary supplementation of κ-carrageenan to the diets of post-larval shrimps at concentration levels of 0.15 g kg^{-1} to 0.30 g kg^{-1} was beneficial and the inclusion of 0.29 g kg^{-1} was optimal. Other refined extracts such as fucoidan from brown seaweed can promote growth and enhance immunological response at 0.05% supplementation with no additional growth benefits observed at higher levels of 0.2%. The optimal level in the present study at 0.03% (equivalent to 0.29 g kg^{-1}) was close enough to that of fucoidan for *P. monodon* (Traifalgar et al., 2009) or for fucoidan for *Penaeus japonicus* larval metamorphic survival (0.05%, Traifalgar et al 2012.) The range was also within that used in a later study by Traifalgar et al., (2013) at 2 g kg^{-1} diet of fucoidan from *Fucus vesiculosus* or κ-carrageenan from *Eucheuma cottonii* for *P. monodon* larvae; however, no significant differences were observed with the control group. The remarkable growth in shrimps fed 0.15 and 0.30 g kg^{-1} κ-carrageenan in the present study could be explained by the acceleration of nutrient absorption of the diet as reflected on their FCE values (**Table 3**). However, this acceleration of absorption might only be true for dietary carbohydrate and lipids but not for dietary protein as reflected on the statistically similar PER values for all dietary groups. Also, the higher level of supplementation of 0.6 g kg^{-1} did not result in any additional growth or efficiency benefits to *P. monodon* in the present study. Generally considered as dietary fiber, κ-carrageenan at higher supplementation resulted in increased dietary fiber content. Potty (1996) explained that fiber structures possibly reduce the accessibility of intestinal enzymes to food nutrients, thereby acting as physical hindrances between nutrients and digestive enzymes in the intestine, resulting in lesser availability. Nakagawa & Montogomery (2007) state that natural compounds that occur in seaweeds such as carrageenans and agar have been observed to block the efficacy of beneficial compounds and inhibit digestion.

Although the optimal inclusion level for growth was estimated to be 0.29 g kg^{-1} in the present study, a higher level of 0.6 g kg^{-1} was required for the highest immunostimulatory effect against acute salinity stress in *P. monodon* in the present study. To our knowledge, this is the first study that evaluated κ-carrageenan as an oral immune promoter against acute salinity stress. In contrast with the few studies in literature which monitored cellular and humoral responses to carrageenan, the present study monitored the organismic level response. Thus, direct comparison would be difficult at best considering differences in the administration of κ-carrageenan, the nature of stress, and the parameters monitored differed across studies. In fish, specifically in common carp *Cyprinus carpio* and grouper *Epinephelus coioides,* a dose of 10-30 mg kg^{-1} of κ-carrageenan via injection has been reported to increase resistance against bacterial infections (Fujiki et al., 1997; Cheng et al., 2007). Chen & He (2019) explained succinctly the close relation of immunity and environmental adaptation in shrimps. According to them, organismal responses to non-biological factors are referred to as environmental adaptations. Although responses to biological factors are often considered part of organismal immunity, these responses also fall into the broader category of environmental adaptations. Thus, following this logic, the stimulation of the shrimp immune system by the dietary κ-carrageenan against acute salinity stress could also influence the immune system of shrimps against pathogen. Engel & Barton (2010) offer the same observation that in shrimp and other aquatic animals, the environmental stress and immune responses are closely related.

Chen et al. (2014) studied the effect of carrageenan on the immune response of white shrimp *L. vannamei in vitro* and *in vivo*. In the *in vivo* study, shrimp received carrageenan via immersion at 200, 400 and 600 mg L^{-1} after 3 h and orally at 0.5, 1.0 and 2.0 g kg^{-1} for 3 weeks showed higher proliferation of haematopoietic tissues (HPTs) together with increases in haemocyte count and other immune parameters. They concluded that carrageenan effectively triggers an innate immunity *in vitro*, and increases mitotic index of HPT, immune parameters, gene expressions and resistance against pathogens *in vivo*. There is an interlinking connection between the attenuation of the effects of environmental stress and immune system in shrimp as Engel & Barton (2010) explained. Also, transcriptomic and proteomics studies in *L. vannamei* have shown that some genes in the humoral immune signaling pathway are differentially expressed in response to environmental stress (Chen & He, 2019). The same investigators have demonstrated that genes differentially expressed in response to environmental stress were associated with various signaling pathways.

Osmotic stress (i.e. acute salinity stress in the context of the present study) also affects the phagocytic activity of *L. vannamei* cells and increases the cellular immune response associated with reactive oxygen species (ROS) (Zhao et al., 2015).

Yeh & Chen (2008) injected white shrimp *L. vannamei* several types of carrageenan at 6 μg g^{-1} and results showed increased resistance against *Vibrio alginolyticus* after 24 h of challenge. The authors hypothesize that the mechanism of the positive effects of carrageenan was (a) that carrageenan receptors exist in macrophages and haemocytes; (b) that carrageenan can be recognized by β-1,3-glucan binding protein (LGBP) or other patter recognition (PRPs) in *L. vannamei*; and (c) that complex (carrageenan and PRP) bind the surface of granular haemocytes leading to the activation of immunity. It could be that the effect of dietary κ-carrageenan in decreasing the mortality rate in *P. monodon* against acute salinity stress in the present study could have led to modification of its cellular and humoral responses in *L. vannamei.* Whether or not it could also serve to protect it against infection by a pathogen remains to be investigated. Currently, we are undertaking a study on the effects of acute salinity stress on the transcriptomic profile of the whole body of *P. monodon* post larvae. The results of this study will elucidate further the molecular mechanism of the protection of κ-carrageenan against acute salinity stress.

In conclusion, κ-carrageenan did not affect the attractability of experimental diets to *P. monodon* indicating that at least the ingredient did not repel the shrimps. Dietary κ-carrageenan resulted in better growth and efficiency performance than did the control diet at inclusion levels of 0.15 and 0.3 g kg^{-1} and there were no additional benefits at higher dietary levels (i.e. 0.45 and 0.60 g kg^{-1}). The optimal inclusion level of dietary κ-carrageenan to the diet of *P. monodon* post larvae was estimated to be 0.29 g kg^{-1} employing second level polynomial regression of WG and SGR data. For the protection of *P. monodon* post larvae against acute salinity stress, the requirement was higher than 0.29 g kg^{-1} for growth; this was at 0.60 g kg^{-1} κ-carrageenan which resulted in the lowest mortality which could be higher since it had not reached a plateau. There was a linear decrease in mortality rate with increased κ-carrageenan level; however, at the highest level of 0.6 g kg^{-1} κ-carrageenan, the benefit of growth promotion would be totally eliminated as a result of increased fiber.

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References

Chen, Y.-H., He, J.-G., 2019. Effects of environmental stress on shrimp innate immunity and white spot syndrome virus infection. *Fish & Shellfish Immunology* 84: 744-755.https://doi.org/10.1016/j.fsi.2018.10.069

Chen, Y.Y., Chen, J.C., Lin, Y.C., Putra, D.F., Kitikiew, S., Li, C.C., Hsieh, J.F., Liou, C.H., Yeh, S.T., 2014. Shrimp that have received carrageenan via immersion and diet exhibit immunocompetence in phagocytosis despite a post-plateau in immune parameters. *Fish & Shellfish Immunology* 36, 352-366.http://dx.doi.org/10.1016/j.fsi.2013.12.004

Chen, L.C., 1990. *Aquaculture in Taiwan*, Fishing News Books, Oxford, UK, 1990, pp. 273-278.

Cheng, J.H., Liao, I.C., 1986. The effect of salinity on the osmotic and ionic concentrations in the haemolymph of *Penaeus monodon* and *P. penicillatus*, in: J.L. Maclean, L. B. Dizon, L.V. Hosillos (Eds.), *The First Asian Fisheries Forum*, Manila, Philippines, Asian Fisheries Society, 1986, pp. 633-636.

Cheng, A.C., Tu, C.W., Chen, Y.Y., Nan, F.H., Chen, J.C., 2007. The immunostimulatory effects of sodium alginate and iota-carrageenan on orange-spotted grouper *Epinephelus coioides* and its resistance against *Vibrio alginolyticus*. *Fish & Shellfish Immunology* 22:197-205. DOI:10.1016/j.fsi.2006.04.009

Declarador, R.S., Serrano Jr., A.E., Corre Jr., V.L., 2014. Ulvan extract acts as immunostimulant against white spot syndrome virus (WSSV) in juvenile black tiger shrimp *Penaeus monodon*. *AACL Bioflux* 7(3):153-161.

Engel, A., Barton, G.M., 2010. Unfolding new roles for XBP1 in immunity. Nature Immunology 11: 365-367.

Fang, L.S., Tang, C.K., Lee, D.L., Chen, I.M., 1992. Free amino acid composition in muscle and haemolymph of the prawn *Penaeus monodon* in different salinities, *Nippon Suisan Gakkaishi* 58(6): 1095-1102.

Fujiki, K., Shin, D., Nakao, M., Yano, T., 1997. Effects of κ-carrageenan on the non-specific defense system of carp *Cyprinus carpio*. *Fisheries Science* 63:934-938. https://doi.org/10.2331/fishsci.63.934

Huang, X., Zhou, H., Zhang, H., 2006. The effect of *Sargassaum fusiforme* polysaccharide extracts on Vibriosis resistance and immune activity of the shrimp, *Fenneropenaeus chinensis*. *Fish & Shellfish Immunology* 20:750-7. https://doi.org/10.1016/j.fsi.2005.09.008

Kautsky, N., Ronnback, P., Tedengren, M., Max, T., 2000. Ecosystem perspectives on management of disease in shrimp pond farming. *Aquaculture* 191: 145–161. https://doi.org/10.1016/S0044-8486(00)00424-5

Kitikiew, S., Chen, J.C., Putra, D.F., Lin, Y.C., Yeh, S.T., Liou, C.H., 2013. Fucoidan effectively provokes the innate immunity of white shrimp *Litopenaeus vannamei* and its resistance against experimental *Vibrio alginolyticus* infection. *Fish & Shellfish Immunology* 34:280-90. https://doi.org/10.1016/j.fsi.2012.11.016

Lauzon, Q.D., Serrano Jr., A.E., 2015. Ulvan extract from *Enteromorpha intestinalis* enhances immune responses in *Litopenaeus vannamei* and *Penaeus monodon* juveniles. ABAH Bioflux 7(1):1-10.

Lazo, J.P., Davis, D.A., 2000. Ingredient and feed evaluation. In: Stickney, R.R. (ed.) *Encyclopedia of Aquaculture*. John Wiley & Sons, Inc., NY, USA. pp. 453-464.

Liu, C.H., Yeh, S.P., Kuo, C.M., Cheng, W., Chou, C.H., 2006. The effect of sodium alginate on the immune response of tiger shrimp via dietary administration-activity and gene transcription. *Fish & Shellfish Immunology* 21:442-452. https://doi.org/10.1016/j.fsi.2006.02.003

Nakagawa, H., Montgomery, L.W., 2007. Algae. In: Nakagawa, H., Sato, M., Gatlin, D.M. (Eds.), *Dietary Supplements for the Health and Quality of Cultured Fish*. CAB International, Oxon, pp. 133-162.

Necas, J., Bartosikova, L., 2013. Carrageenan: a review. *Veterinarni Medicina* 58, 187-2015. DOI:10.17221/6758-VETMED

Peñaflorida, V.D., Golez, N.V., 1996. Use of seaweed meals from *Kappaphycus alvarezii* and *Gracilaria heteroclada* as binders in diets for juvenile shrimp *Penaeus monodon*. *Aquaculture* 143, 393-401. https://doi.org/10.1016/0044-8486(96)01282-3

Potty, H.V., 1996. Physico-chemical aspects, physiological functions, nutritional importance and technological significance of dietary fibres—a critical appraisal. Journal of Food Science & Technology 33: 1-18.

Raa, J., 1996. The use of immunostimulatory substances in fish and shellfish farming. *Reviews in Fisheries Science* 4:229-88. https://doi.org/10.1080/10641269609388587

Sakai, M., 1999. Current research status of fish immunostimulants. *Aquaculture* 172, 63-92. https://doi.org/10.1016/S0044-8486(98)00436-0

Serrano Jr., A.E., Declarador, R.S., 2014. Growth performance of black tiger shrimp *Penaeus monodon* larvae fed diets supplemented with ulvan. *ABAH Bioflux* 6(2), 173-180.

PSA (Philippine Statistics Authority), 2020. Fisheries Situation Report (January to March 2020). Quezon City. Philippines. https://psa.gov.ph/sites/default/files/attachments/ird/specialrelease/SR_Q12020_FSD.pdf

Suresh, A.V., Kumaraguru vasagam, K.P.K, Nates, S., 2011. Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, *Litopennaeus stylirostris*, fed diets formulated with high levels of poultry byproduct meal. *Aquaculture*, 319: 132-140. https://doi.org/10.1016/j.aquaculture.2011.06.039

Traifalgar, R.F.M., Koshio, S., Ishikawa, M., Serrano, A.E., Corre, V.L., 2012. Fucoidan supplementation improves metamorphic survival and enhances vibriosis resistance of *Penaeus japonicus* larvae. *Journal of Fisheries and Aquaculture* 3 (1): 33–36. http://dx.doi.org/10.9735/0976-9927.3.1.33-36

Traifalgar, R.F.M, Corre, V.L., Serrano Jr., A.E., 2013. Efficacy of dietary immunostimulants to enhance the immunological responses and vibriosis resistance of juvenile *Penaeus monodon*. *Journal of Fisheries and Aquatic Sciences* 8(2):340-54. DOI:10.3923/jfas.2013.340.354

Traifalgar, R.F.M., Serrano, A.E., Corre, V., Kira, H., Tung, H.T., Michael, F.D., Kader, M.A., Laining, A., 2009. Evaluation of dietary fucoidan supplementation effects on growth performance and vibriosis resistance of *Penaeus monodon* postlarvae. *Aquaculture Science*. 57(2): 167-174. https://doi.org/10.11233/aquaculturesci.57.167

Van Doan, H.V., Hoseinifar, S.H., Esteban, M.A., Dadar, M., Nang Thu, T.T., **2019.** Mushrooms, seaweeds, and their derivatives as functional feed additives for aquaculture: An update view. *Studies in Natural Products Chemistry*, 41-89. https://doi.org/10.1016/B978-0-444-64185-4.00002-2

Wang, L.U., Chen, J.C., 2005. The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* at different salinity levels. *Fish & Shellfish Immunology* 18: 269-278. https://doi.org/10.1016/j.fsi.2004.07.008

Yeh, S.T., Chen, J.C., 2008. Immunomodulation by carrageenans in the white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. *Aquaculture* 276, 22-28. doi:10.1016/j.aquaculture.2008.01.034

Yeh ST, Chen JC., 2009. White shrimp *Litopenaeus vannamei* that received the hot-water extract of *Gracilaria tenuistipitata* showed earlier recovery in immunity after a *Vibrio alginolyticus* injection. *Fish& Shellfish Immunology* 26: 724-30. https://doi.org/10.1016/j.fsi.2009.02.025

Zhao, Q., Pan, L., Ren, Q., Hu, D., 2015. Digital gene expression analysis in hemocytes of the white shrimp *Litopenaeus vannamei* in response to low salinity stress. *Fish & Shellfish Immunology* 42: 400-407. https://doi.org/10.1016/j.fsi.2014.11.020