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Synergistic effects of *Quillaja* saponin and κ-carrageenan on the growth and resistance against hyposalinity change in the black tiger shrimp *Penaeus monodon* post larvae

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Key Words: immunostimulants, *Kappaphycus alvarezii*, *Quillaja saponaria*, receptor recognition, membrane permeabilization

Abstract

The present study aimed to determine whether or not combining *Quillaja* saponin (*QS*) and κ-carrageenan (*KC*) as feed supplements could have additive or synergistic effect as growth-promoters and/or immune enhancers against hyposalinity stress in *Penaeus monodon* post larvae. Three separate experiments were done: (a) a random three days attractability test; (b) a 30-day feeding trial to determine optimal inclusion of combined *QS+KC*; and (3) a 24 h acute hyposalinity stress test following 30-day feeding five experimental diets. Attractability results showed that the *QS+KC* very briefly attracted the shrimps for 2 min ($p<0.05$) but became non-significant after longer periods. In the feeding trial, 5 experimental diets containing graded levels of *QS+KC* (1:1), namely, 0.0, 0.2, 0.4, 0.6, and 0.8 g kg^{-1} were fed to groups of shrimps. Results showed that the final average body weight (FABW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) values of shrimps in the supplemented groups were significantly better than those of the control group; the values of those in 0.2 and 0.8 g·kg^{-1} *QS+KC* groups were not significantly different from those of the control group. Feed intake (FI) and protein efficiency ratio (PER) values were not affected by the supplementation ($p>0.05$). Optimal inclusion level of dietary *QS+KC* was determined to be 0.4 g·kg^{-1}. After the feeding trial, shrimps were transferred from 24 ppt salinity media to 2 ppt. All *QS+KC*-fed groups exhibited significantly lower cumulative mortality rate than that of the 0.0 *QS+KC* group ($p<0.05$). The determined level of *QS+KC* that elicited the lowest cumulative mortality rate was 0.5 g·kg^{-1}. In conclusion, the study demonstrated that the combined dietary *Quillaja* saponin and *kappa carrageenan* acted synergistically in the promotion of growth and feed efficiency and in the enhancement of immune response against acute hyposalinity stress.

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Introduction

The black tiger shrimp, *Penaeus monodon*, can adapt to a wide range of environmental salinity levels i.e., a euryhaline species. This phenomenon could reflect its life cycle. It begins as planktonic larvae in coastal waters, enters estuarine nursery grounds as post larvae and goes back to the ocean as an adult (Wickins, 1976). However, in open aquaculture system such as that in ponds in which the water medium could be shallow enough to be perturbed by the local climate e.g., under full sunshine where it could elevate the salinity or heavy rain that could cause salinity to suddenly decrease. Prolonged exposure to salinity outside its optimal range of 10-20 ppt (Fang et al., 1992) may cause stress and weaken the immune system making them highly vulnerable to opportunistic pathogens. There are evidence for metabolic changes and immune depression in shrimps in response to variations in salinity (Wang & Chen, 2005; Joseph & Philip, 2020; Shekhar et al., 2013). There is a need to strengthen the innate immune system of *Penaeus monodon* by using immunostimulants.

As immunostimulants, the native and commercial carrageenans from *Kappaphycus alvarezii* contain various phytochemicals that include sugar derivatives of saponins (Suganya et al., 2016). Considering that they exist together in some natural compounds, at least very little or no antagonism is expected when combining κ-carrageenan and saponin in the diet of aquaculture animals. The additive and synergistic effects of phytochemicals in seaweeds have been proposed as being responsible for their potent antioxidant and anti-cancer activities (Nagarani & Kumaraguru, 2012). Both native and commercial carrageenans exhibited better antioxidant activities such as total antioxidant capacity, hydroxyl radical scavenging activity, nitric oxide radical scavenging activity, diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity and reducing power assay (at the maximum concentration of 100 µg/ml carrageenans (Suganya et al., 2016). The relationship of *Quillaja* saponin and κ-carrageenan could also be traced in their ability to counteract each other in studies in mice.

Saponin-based adjuvants have the unique ability to stimulate the cell-mediated immune system, as well as to enhance antibody production, and have the advantage that only a low dose is needed for adjuvant activity (Oda et al. 2003). Many saponins display significant antinociceptive (i.e. inhibits the sensation of pain), anti-inflammatory and antipyretic activities (reduction of fever) possibly due to their nonglycosidic moiety, the sapogenin, but also many diverse activities have also been reported such as antiallergic, antifungal, analgesic and others (Hostettmann & Marston, 1995; Milgate & Roberts, 1995; Lacaille-Dubois & Wagner, 1996; Francis et al., 2002).

Previously, we demonstrated the positive effects of κ-carrageenan and *Quillaja* saponin separately on enhancing the growth and resistance to sudden hyposalinity shock on the black tiger shrimp (Jumah et al., 2020a, b). Despite the positive effect of a single immunostimulant supplementation in aquaculture, recent studies demonstrated that a combination of two or more immunostimulants can have better immunostimulatory effect than a single application in animals (Tiengtam et al., 2015; Swanson et al., 2002; Piaget et al., 2007). Thus, the present study aimed to investigate the effects of combined dietary saponin and κ-carrageenan on the growth and resistance of *Penaeus monodon* against acute hyposalinity stress and find out whether or not these bioactive compounds would act synergistically or additively as a dietary supplement in the diet of *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 a commercial hatchery about 12 km from the wet laboratory of the university. The shrimp larvae which were certified White Spot Syndrome Virus (WSSV) and *Vibrio parahaemolyticus*-free, were transported in plastic bags half-filled with sea water into which oxygen gas was blown. In the laboratory on arrival, shrimps were slowly acclimatized to ambient temperature and salinity of the water in a 250-L fiberglass holding tank. The shrimps were fed with the basal (i.e., control) diet for 5 days prior to distribution into the experimental containers.

Diet preparation

Five diets containing various concentrations of combined purified *Quillaja* saponin, *QS,* (Sigma product no. S-4521 extracted from the bark of the soap tree *Quillaja saponaria,* Sigma-Aldrich Corp., MO, USA) and κ-carrageenan, *KC*, (Shemberg Mktg Corp., Cebu, Philippines) were prepared. The combined *QS and KC* was maintained to a constant ratio of 1:1 and the dietary treatments contained graded levels of this combination, namely, 0.0, 0.2, 0.4, 0.6 and 0.8 $g \cdot kg^{-1}$ diets (**Table 1**). Dry ingredients were sieved and weighed individually, and were thoroughly mixed in a large transparent plastic bag. Liquid ingredients such as lecithin, fish oil and ethanol-dissolved BHT and 5-hydroxytryptophan were added next. Purified saponin and κ-carrageenan were dissolved in distilled water and added to the mixture. Gelatinized high-grade flour (i.e., cooked in distilled water to the desired consistency) was added last and the moist mixture was manually kneaded inside the plastic bag into dough. Prior to oven drying, the dough was steamed for 15 min, rolled out thinly on a metal tray onto which small square size portions were sliced in place using a knife. Oven drying was done for 24 h at 60℃ until the moisture reached about <10 %. The oven-dried experimental diets were collected in square-shaped pieces, put in plastic bags and stored at -20℃ until use.

Diet attractability test

Three separate attractability tests were conducted in three replicates using rectangular glass tanks with multiple chambers (Suresh et al., 2011) and the placement of the diet changed with each repeated test. Each tank consisted of 3 major chambers (an acclimatization chamber, a middle chamber, and a feeding chamber) that were separated with glass partitions. The feeding chamber consisted of 5 sub-chambers measuring 6 x 5 cm (**Figure 1**).

Each customized glass tank (90 x 50 x 30 cm L, W and H, respectively) consisted of an acclimation chamber at one end and feeding chambers at the other end separated by a removable glass partition. All assessments were conducted at the same time of the day. Diet attractability tests were performed on the 5 experimental diets in three simultaneous runs. Each diet arrangement in the feeding chamber was triplicated to ensure its consistency. Fifteen randomly selected fasted-shrimps were acclimatized for 1 h in the acclimatization chamber, then 2 g of each experimental diet were placed separately in each of the feeding sub-chambers. Following the lifting of the glass partition, the number of shrimps that entered each of the feeding chambers was recorded at 1-, 2-, 5-, 10- and 15-min. Diet attractability was expressed as percent of shrimp that were inside a particular feeding chamber containing a particular experimental diet after following the lifting of the glass partition.

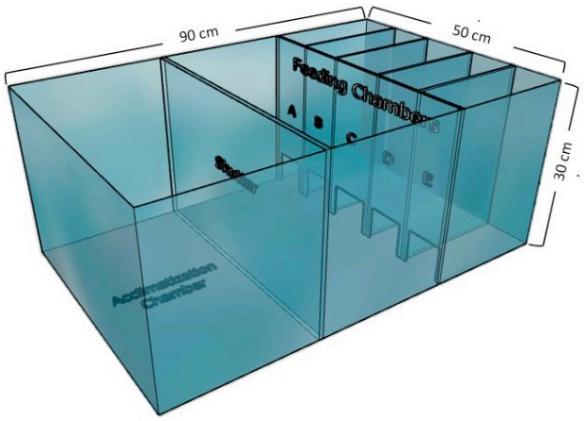

Figure 1 Schematic diagram of the tank used for the attractability test.

Table 1 Feed composition and proximate analysis of experimental diets containing the combined *Quillaja* saponin (*QS*) and κ-carrageenan (*KC*) for *Penaeus monodon* post larvae growth trial (g·kg^{-1} diet) for 30 days.

Ingredients	*0*	*0.2*	*0.4*	*0.6*	*0.8*
Danish fish meal	200.0	200.0	200.0	200.0	200.0
Shrimp meal	340.0	340.0	340.0	340.0	340.0
Squid meal	210.0	210.0	210.0	210.0	210.0
CMC	34.7	34.5	34.3	34.1	33.9
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
5-HTP	0.1	0.1	0.1	0.1	0.1
QS+KC (1:1)	0.0	0.2	0.4	0.6	0.8
Total	1000.0	1000.0	1000.0	1000.0	1000.0
Proximate analysis (%, dry weight basis)					
Crude Protein	48.40	48.30	48.51	48.50	48.25
Crude Fat	11.00	11.11	11.14	11.12	11.08
Crude Fiber	3.20	3.23	3.25	3.57	3.68
Moisture	9.80	9.80	9.09	9.45	9.52
Ash	14.50	14.40	14.5	14.30	14.19
NFE	13.10	13.16	13.51	13.06	13.28

[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, inert carrier q.s added 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.

Feeding trial experiment

Post larval shrimps of uniform sizes (average body weight of 10.0 mg) were distributed into 15 experimental containers in a completely randomized design (CRD) in a recirculation system. The recirculation system was consisted of a reservoir, the water from which was pumped onto an elevated filter (placed on a 7 ft-platform), a 50-L capacity container filled with sand and gravel; the water drained onto a lower biological filter container by gravity

which contained sterilized empty oyster shells. The water coming from the biological filter was distributed into a pipe system by gravity into each of the experimental units. Feeding was done four times daily at 0700, 1000, 0100, and 1430 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 practically *ad libitum* starting at the basal rate of 30% body weight for the first 2 weeks and 20% for the last 2 weeks. Daily ration was closely monitored at each feeding time and adjusted accordingly i.e., either to stop feeding when no feeding activity was detected or to add more feeds when feeds were totally consumed in a short period of time. Water quality indices 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 indices

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

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

$$SGR\ (\%\ daily) = 100*(\ln FBW - \ln IBW) / D$$

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

$$FCR = \text{feed offered (g)} / \text{wet weight gain (g)}$$

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

$$SURV\ (\%) = 100*(\text{final number of shrimp} / \text{initial number of shrimp})$$

At the start and termination of the trial, all shrimps in each feeding unit were carefully collected with a fine-meshed scoop net, the bottom of which was blotted onto a clean paper towel, and the shrimps were placed into a small clear plastic cup and quickly weighed. Shrimps were weighed by batch using a digital top-loading analytical balance.

Acute salinity stress test

At the end of 30-day feeding trial, 225 shrimps were transferred from 24 ppt into 2 ppt medium for acute salinity challenge test. There were 15 units of 10-L plastic container laid out in a completely randomized design (CRD) in an indoor static water system. Each container was provided with aeration and continuously fed every 4 h until the end of the 24 h study. A shrimp was considered dead when it did not respond when poked with a glass rod; mortality was monitored every 15 min for 1 h, then every hour for 4 h, followed by every 4 hours for 12 h. Water parameters such as pH, salinity, DO and temperature were measured during the experiment.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, version 23). Percent data were transformed into arcsine values prior to analysis. 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. Data were 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.

Table 2 Water quality indices during feeding trial of the black tiger shrimp *P. monodon* post larvae fed with various dietary levels of combined *Quillaja* saponin and κ-carrageenan (1:1) for 30 days.

Water Quality Parameter	*Treatment (g/kg)*				
	0	*0.2*	*0.4*	*0.6*	*0.8*
Salinity (‰)	23.9 ± 2.9	23.9 ± 2.9	24.0 ± 3.0	24.0 ± 3.0	23.9 ± 2.9
Temp. (℃)	28.4 ± 3.5	28.4 ± 3.5	28.4 ± 3.5	28.4 ± 3.5	28.4 ± 3.5
pH	8.3 ± 1.0	8.3 ± 1.0	8.3 ± 1.0	8.3 ± 1.0	8.3 ± 1.0
DO (ppm)	6.8 ± 0.8	6.8 ± 0.8	6.9 ± 0.9	6.9 ± 0.9	6.9 ± 0.9
NH_3/NH_4^+ (ppm)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
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)	3.8 ± 1.1	3.8 ± 1.1	3.8 ± 1.1	3.8 ± 1.1	3.8 ± 1.1

Results of the attractability tests showed that the diets containing *QS+KC* significantly affected the attractability for the first two min ($p<0.05$) beyond which their proportion on each feeding chamber did not differ significantly ($p>0.05$).

Table 3 Attractability of shrimp *P. monodon* post larvae to the experimental diets containing various levels of *Quillaja* saponin and κ-carrageenan diets (1:1).

Treatment (QS+KC g·kg^{-1})	*Time (min)*				
	1	*2*	*5*	*10*	*15*
0.0	3.70±1.52^b	5.56±2.48^b	12.22±3.04^a	24.81±3.97^a	28.15±4.41^a
0.2	10.74±1.45^a	12.22±2.36^a	20.37±3.58^a	32.22±5.36^a	41.85±6.26^a
0.4	11.85±1.77^a	14.81±2.61^a	24.07±4.57^a	33.70±7.21^a	42.22±8.87^a
0.6	15.56±3.89^a	16.67±3.85^a	24.07±5.55^a	34.07±4.99^a	47.04 ±8.34^a
0.8	16.67±4.30^a	17.78±4.58^a	27.78±6.33^a	36.67±8.46^a	50.37±11.61^a

Means having the same superscripts are not significantly different from each other ($p>0.05$).

Growth trial

The growth trial period of 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 showed that survival rate, FI and PER values were not significantly different among the groups at the end of the 30-day feeding trial (**Table 4**). FABW, WG and SGR values exhibited similar trends in which the values were significantly highest in the 0.4 g·kg^{-1} *QS+KC* treatment ($p<0.05$) followed by 0.2 and 0.4 g·kg^{-1} which were not significantly different from each other ($p>0.05$) and the lowest values in the 0.0 and 0.8 g·kg^{-1} *QS+KC* treatments which were statistically similar ($p>0.05$).

Table 4 Growth performance of the black tiger shrimp *Penaeus monodon* fed with diets containing different concentrations of mixed dietary *Q*-saponin (*QS*) and κ-carrageenan (*KC*) for 30 days.

Index	*Treatment (g/kg of QS+KC)*				
	0.0	*0.2*	*0.4*	*0.6*	*0.8*
IABW (mg)	10.0 ± 0.0	10.0 ± 0.00	10.0±0.0	10.0 ± 0.0	10.0 ± 0.0
FABW (mg)	45.9 ± 0.5^{c}	51.5 ± 0.9^{b}	59.0 ± 0.8^{a}	54.7 ± 1.0^{b}	47.9 ± 1.2^{c}
WG (mg)	35.9 ± 0.5^{c}	41.5 ± 0.9^{b}	49.0 ± 0.8^{a}	44.7 ± 1.0^{b}	37.9 ± 1.2^{c}
FI (mg)	230.0±24.0^{a}	235.9 ± 7.6^{a}	276.2 ± 8.3^{a}	250.5±11.0^{a}	250.8±22.1^{a}
SGR(%·d^{-1})	4.56 ± 0.05^{c}	5.1 ± 0.1^{b}	6.0 ± 0.2^{a}	5.4 ± 0.1^{b}	4.8 ± 0.1^{c}
PER	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
FCR	2.1 ± 0.0^{c}	1.8 ± 0.0^{b}	1.5 ± 0.1^{a}	1.7 ± 0.0^{b}	2.0 ± 0.1^{c}
SURV (%)	80.0 ± 8.0^{a}	73.3 ± 2.7^{a}	80.0 ± 2.3^{a}	73.3 ± 3.5^{a}	84.0 ± 8.3^{a}

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.

Optimal inclusion rate of the combined dietary *QS+KC* was determined by fitting separately the WG, SGR, and FCR values into quadratic regression equation to be 0.4 g kg^{-1} *QS+KC* (**Figure 2**).

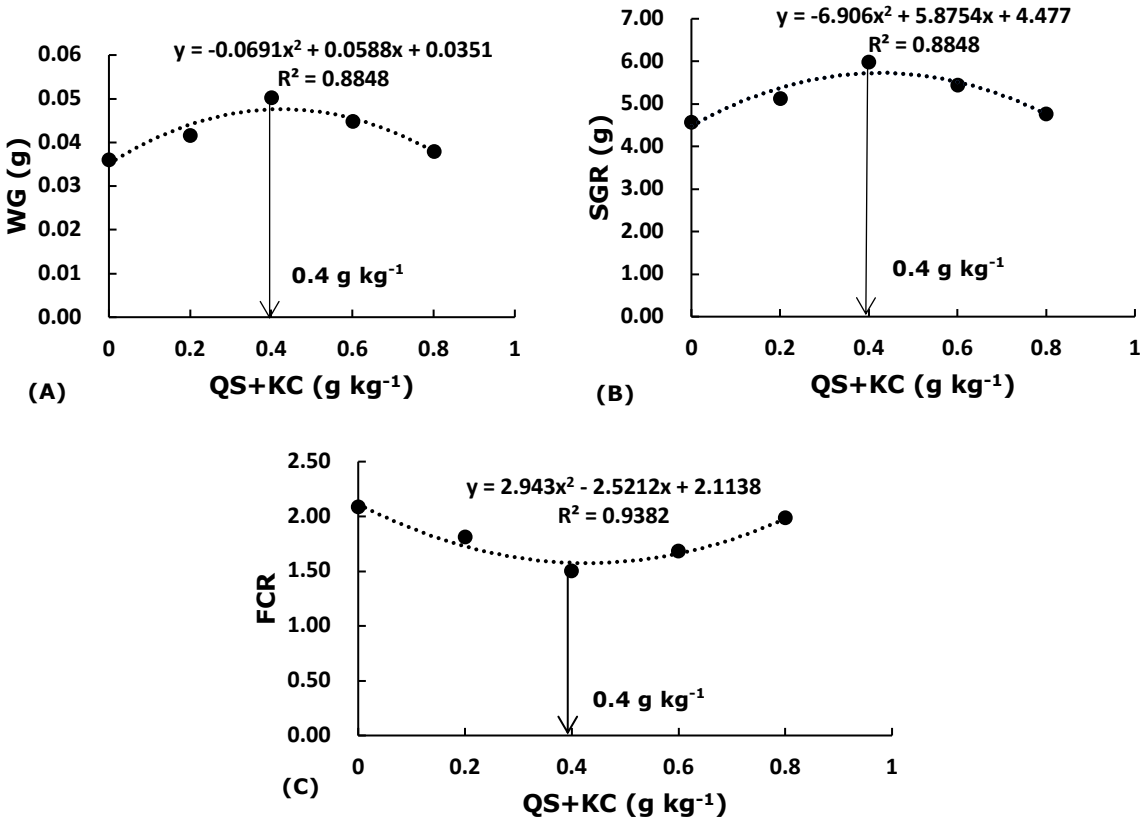

Figure 2 Optimal level of dietary *QS+KC* determined by fitting weight gain (A), specific growth rate (B), and feed conversion ratio (C) data into a quadratic regression model.

Low salinity challenge test

Results of the acute hyposalinity stress test showed that shrimps fed the control diet (0.0 g·kg^{-1} diet exhibited significantly the highest cumulative mortality rate of 97.8% followed by those fed *0.6, 0.8, 0.2* g·kg diet^{-1} 24 h following transfer from 24 ppt to 2 ppt medium; these three groups were not significantly different from each other (p>0.05) (**Figure 3** and **Table 5**). The control group exhibited significantly the lowest survival rate of 44.4% among the dietary treatments (p<0.05).

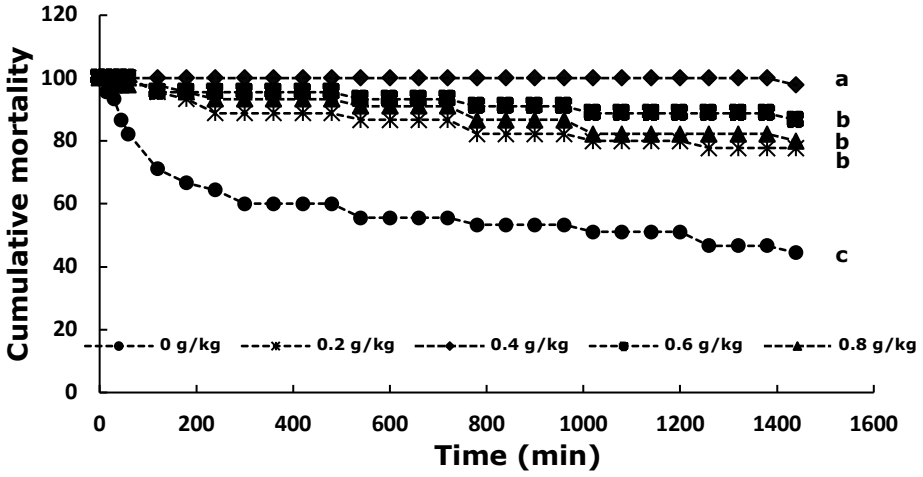

Figure 3 Cumulative mortality of black tiger shrimp post larvae in the acute hyposalinity challenge test following feeding diets containing mixed *Quillaja* saponin and *kappa* carrageenan inclusions for 30 days.

Table 5 Final survival rate of shrimps after 24 h in 2 ppt salinity challenge test.

Treatment (QS+KC g/kg)	*Cumulative mortality rate (%)*
0.0	55.6 ± 1.2^{c}
0.2	22.2 ± 0.5^{b}
0.4	2.2 ± 0.0^{a}
0.6	13.3 ± 0.3^{b}
0.8	20.0 ± 0.3^{b}

Means having the same superscripts are not significantly different from each other (p>0.05).

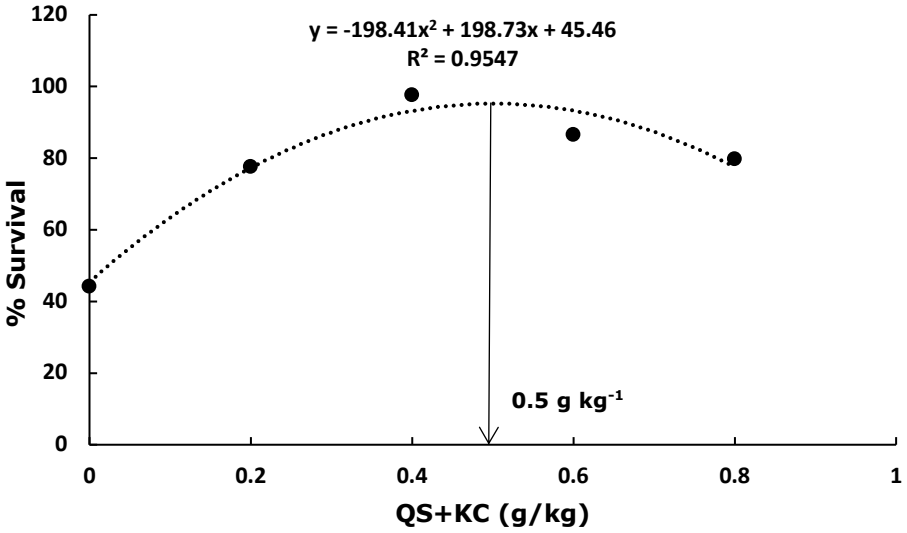

Figure 4 Quadratic estimation of the optimal level of combined dietary *Quillaja* saponin and κ-carrageenan that elicited the highest percental of survival after transfer from 24 ppt to 2 ppt medium for 24 h.

Discussion

In the previous study, dietary inclusion of κ-carrageenan alone did not enhance nor inhibit the attractability of the diet to *Penaeus monodon* post larvae (Jumah et al., 2020a) but dietary saponin did increase it (Jumah et al., 2020b). Combining the two supplements in the current study slightly increased the attractability very quickly (i.e., 2 min) and eventually, no significant differences were observed between treatments (p>0.05). In this case, it was perhaps the presence of *KC,* which did not improve attractability in the previous study that neutralized the attractability effect of the *QS*.

The negligible effect that *QS* and *KC* exhibited on diet attractability was manifested on the FI being unaffected among dietary groups. Despite this, there were significant differences in the growth performance of the groups. The effect was specifically noted on the feed efficiency, specifically FCR, since PER was also unaffected. At 0.4 g·kg^{-1} *QS+KC*, FCR value was significantly the lowest (i.e., most efficient) and growth rate was significantly the highest in terms of WG and SGR. We hypothesize that the dietary *QS* affected positively the growth and feed efficiency in the present study while KC positively affected the immunity in the hyposalinity challenge test. It has been demonstrated much earlier that (a) saponin can permeabilize cell membranes without destroying them (Jacob et al., 1991; Petterson et al., 1999; Onning et al., 1996; Price et al., 1987) and that (b) the nutrients were absorbed more readily by the permeabilized gut membrane (Serrano, 2013). We hypothesize further that further increases in the concentration of the permeability agent (saponin in the present study) would not have any further effect on the membrane. This might be the reason why from the peak of maximum growth and efficiency at 0.4 g·kg^{-1} QS+KC, the values significantly decreased at 0.6 g·kg^{-1} QS+KC and further significantly decreased at 0.8 g·kg^{-1} dietary level. In addition, the permeabilization of the gut membrane may also lead to activation of both digestive and metabolic enzymes as observed by Serrano (2013) in common carp that led to more nutrient conversion.

In the case of the hyposalinity challenge test in the present study, the level that resulted in the maximal reduction in the cumulative mortality was 0.5 g·kg^{-1} *QS+KC* (1:1). Since at the optimal level of 0.5 gkg^{-1}, in which QS and KC were 2.5 g·kg^{-1} each, this was remarkably lower than the estimated optimal level of *QS* and *KC* supplemented singly in the previous studies, namely, 0.33 gkg^{-1} for *QS* only and 0.6 gkg^{-1} for *KC* only; clearly,

synergistic effects were at work. The protective effect could be attributed more to the action of *KC* on shrimp immune system as demonstrated in the report of Yeh & Chen (2008) that (a) carrageenan receptors exist in macrophages and haemocytes; (b) that carrageenan can be recognized by β-1,3-glucan binding protein (LGBP) or other pattern recognition proteins (PRPs) in *Litopenaeus vannamei*; and (c) that the complex (i.e., carrageenan and PRP) bind the surface of granular haemocytes leading to the activation of immunity. In invertebrates like shrimps, responses to environmental stress or to disease pathogens are through nonspecific defense mechanisms since they do not possess other specific defenses.

In conclusion, combined dietary κ-carrageenan and *Quillaja* saponin very shortly affected attractability but eventually did not differ significantly at a later period. Combined dietary *Quillaja* saponin and κ-carrageenan supplements resulted in better growth and efficiency than did the control diet despite the similarity in the amount of feed intake (FI). At the inclusion level of 0.4 $g \cdot kg^{-1}$ *QS+KC* (1:1), maximal growth and efficiency were observed and there was no additional benefit at lower or higher dietary levels (i.e., 0.2 or 0.6 and 0.8 $g \cdot kg^{-1}$ *QS+KC*). The dosage that elicited maximal protection against hyposalinity exposure was estimated to be 0.5 $g \cdot kg^{-1}$ *QS+KC* (1:1). Both the effects on growth, feed efficiency and resistance against hyposalinity challenge of the combined QS and KC exhibited synergistic effects.

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