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GROWTH ASSESSMENT OF VARIOUS FORMULAE OF ESSENTIAL MINERALS AND TRACE ELEMENTS ON WHITELEG SHRIMP AT DIFFERENT SALINITIES

Phuong Viet Do[1], Suong Thao Nguyen[2], Tao Tai Chau[3], Loan Nguyen TK[4], Audric Touchet[1], Gaetan Gutter[1], Philippe Mahl[1], Hoang Phan[1,✉]

[1]*Aquaculture Division, Virbac, 13e rue LID - BP 27 06511 Carros cedex, France*
[2]*Fish Pathology Department, Faculty of Fisheries, Nong Lam University, Quarter 6, Linh Trung, Thu Duc City, Ho Chi Minh City, Vietnam*
[3]*Department of Coastal Aquaculture, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, Ninh Kieu District, Cantho Province, Vietnam*
[4]*Pharmaceutical Development Lab, Virbac RDL Lab, Song May IP, Trang Bom District, Dongnai Province, Vietnam*

✉To whom correspondence should be addressed. E-mail: hoang.phan.phd@gmail.com

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SUMMARY

Whiteleg shrimp (*Litopenaeus vannamei*) is now the main species cultivated in various geographic locations. Whiteleg shrimp have a wide range of salinity tolerance, but a matter of deficiency of minerals and trace elements need to be taken care of when shrimp are grown in low saline area. It may induce higher mortality and slower growth performances of shrimp. Two stages of shrimp juveniles were subsequently tested with different formulae of a liquid nutraceutical of essential minerals and trace elements. Each stage was performed in a 28-day trial with follow-up parameters of shrimp growth indices and environmental changes throughout the tests. The use of mineral supplementation in whiteleg shrimp diet in low salinity area significantly increased growth performance, survival rate and color intensity. This study has demonstrated the efficacy of a liquid form of mineral supplement product administered by top-coating onto the feed to shrimp during 4 weeks of grow-out at 5‰ salinity, but the application was dependent on formula and dose. Further research on application regime using this liquid mineral product should be performed to optimize a protocol for a whole culture crop as different stages of shrimp may require different supplementation doses.

Keywords: growth, mineral, molting, survival, trace element, whiteleg shrimp

INTRODUCTION

Over the past decades, shrimp farming has widely spread through the tropical belt, from Latin America to Asia. Whiteleg shrimp (*Litopenaeus vannamei*) is now the main species cultivated due to its wide tolerance to salinity (0.5 to 40‰) and its higher growth performance compared to its relative black tiger shrimp (*Penaeus monodon*). In Vietnam, whiteleg shrimp have been popularly reared since it's first introduction in 2000. According to the Ministry of Agriculture and Rural Development (2019) of Vietnam, whiteleg shrimp production was estimated at 243,000 tons in the first nine months of 2019, with a culture area of 70,700 ha. The most common type of rearing system for whiteleg shrimp is with intensive and super intensive densities. Sometimes, it is reared in low salinity water. This rearing model becomes more popular in the world.

Even if whiteleg shrimp have a better tolerance to low salinity water (<10‰), some challenges need to be overcome when practicing farming in low saline area. A lack of even one major mineral may induce higher mortality and lower growth performances (Saoud *et al.*, 2003, Valenzuela-Madrigal *et al.*, 2017). Low salinity may be chronic – due to the environment of the farm mainly induced by a remoteness of the farming area to the acute seashore, due to episodes of heavy rain, flooding, or in the less protected farming area including drastic shift in the ionic balance. Adding minerals into the water to balance the ionic profile is now commonly known by farmers. Nevertheless, the solubility of these salts, hence their bioavailability, may be lower due to the effect of low salinity (Chitra *et al.*, 2017).

The research on mineral supplementation in the diet of whiteleg shrimp under low salinity condition becomes prioritized to evaluate the growth performance and survival rate. Minerals play an important role in osmotic regulation and shrimp molting. The deficiency of mineral content in low salinity water may cause "soft shell syndrome" and/or molting issues, or evenly high mortality. As many mineral-supplied products are available in the market, the evaluation of quality should be conducted in order to determine the effect of mineral supplements in diet on growth performance, molting, and survival rate. Thus, the study on the effects of Formula 1 and Formula 2 products on whiteleg shrimp rearing at two salinity levels i.e. 5‰ and 15‰ was conducted.

MATERIALS AND METHODS

Two stages of whiteleg shrimp

The study was performed on two stages of *L. vannamei* shrimp juveniles: the first stage (FS, 1.57 ± 0.12 g/individual) and the second stage (SS, 9.8 ± 1.2 g/individual). Prior to being distributed to experimental aquaria, shrimp were screened by PCR for white spot syndrome virus (WSSV) and *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) to ensure the shrimp were free from those specific pathogens (Sirikharin *et al.*, 2014, Lo *et al.*, 1996). Experimental shrimp were in good health when being distributed to experimental fiberglass tanks.

FS juveniles were procured from National Breeding Center for Southern Marine Aquaculture, Vung Tau, Vietnam and pre-grown for acclimatation. SS juveniles had been nursed for approximately 2 months in a recirculation system at the shrimp hatchery/nursery of College of Aquaculture and Fisheries, Can Tho University, Vietnam. Shrimp were acclimatized to experimental salinities (15‰ and 5‰) by stepwise decrease at the rate of 2‰ per day from the original salinity by the addition of freshwater. The juveniles were then randomly stocked in experimental fiberglass tanks (1 m^3 circular form). The two stages i.e. FS and SS were performed separately with control treatments (see details below).

Formulation, rearing model and conditions

The study was performed with 16 treatments in total with 4 formulae tested. Each treatment was carried out in triplicate. The formulation was set with a core mixture of ingredients (named as Calciphos, manufactured by Virbac Vietnam) i.e. formula 1 (major ions of calcium, phosphate, magnesium, sodium, manganese and zinc); formula 2 was added with a salt combination of potassium (K), iron (Fe) and selenium (Se), whilst formulae 3 and 4 were added with a salt of K and Fe respectively, as the modified versions of formula 1. Commercial feed pellets (protein 40–42%, lipid 5.5–6%, fiber 3%, calcium 2.3%, phosphorus 1–1.5%, moisture 11% and minimum energy ~2,600 kcal/kg) were used with top-coating of the formulae accordingly. Final top-coating was applied without fish oil for FS, or with fish oil for SS (20 mL/kg feed). The control feed pellets were accordingly prepared without formulae.

For FS, there were 10 treatments with all 4 formulae. Five of them (4 formulae and a control) were carried out at 5‰ salinity, whereas the other five treatments were performed at 15‰ salinity. All FS treatment tanks were kept indoor (Fig. 1A) with continuous aeration and water exchange of 50–70% depending on the water quality i.e. nitrite (NO_2^--N) and total ammonia-nitrogen (TAN, NH_4^+/NH_3). Juveniles were stocked at a density of 60 individuals per tank and fed five times per day until satiation. In each tank, there were 10 mesh cages to keep 10 individuals (1 shrimp/cage of 20 cm in diameter) for molting observations. The remaining 50 individuals were cage-free for other follow-up parameters. Throughout the study period of 28 days, the amount of feed used, water temperature, dissolved oxygen, pH, NO_2^--N and TAN were recorded every day.

For SS, there were 6 treatments performed on 2 formulae (i.e. 1 and 2 tested at 5‰ salinity), and a normal feed control at 5‰ and 15‰ salinities to be aligned with the FS. The formulae were applied at 2 different doses i.e. 5 and 10 mL/kg feed to further examine the possible effect. Tanks in this stage were placed outdoor with a HDPE cover (opened 6 hours daily from 6 am to 12 pm and during feeding) (Fig. 1B). In 800 L of clean treated water, shrimp were kept separated in small cages at 30 individuals per tank. The water was exchanged by 20% with new water every 2 days. Shrimp were fed 4 times per day manually to satiation. During 28 experimental days, water temperature and pH were measured twice a day at 8 am and 3 pm, and measurement of NO_2^--N and TAN was performed twice a week.

Figure 1. Experimental set-up used for testing various formulae at defined salinities for (A) first stage shrimp and (B) second stage shrimp.

Measurement of growth parameters of shrimp

Shrimp in each tank were weighed at the beginning and at the end of experiment after 28 days. Growth performances of both shrimp stages including weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR) are calculated as follows:

$$\text{WG (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{DWG (g/day)} = \frac{\text{Final weight} - \text{Initial weight (g)}}{\text{Days of culture (day)}}$$

$$\text{SGR (\%/day)} = \frac{\ln\text{(Final weight)} - \ln\text{(Initial weight)}}{\text{Days of culture (day)}} \times 100$$

For bigger shrimp of SS, initial and final lengths of shrimp were measured for calculating daily length gain (DLG):

$$\text{DLG (cm/day)} = \frac{\text{Final length} - \text{Initial length (cm)}}{\text{Days of culture (day)}}$$

During the experiment, feed utilization/intake was recorded in all treatments for the calculation of feed conversion ratio (FCR):

$$\text{FCR} = \frac{\text{Total feed intake (g)}}{\text{Biomass gain (g)}}$$

All dead shrimp were quickly removed from tanks and recorded for survival rate (SR) calculation. Shrimp in small mesh cages were checked for molting 4 times per day, and cumulative molting rate (CM) is calculated as below:

$$\text{SR (\%)} = \frac{\text{Total number of shrimp at harvest}}{\text{Initial number of shrimp}} \times 100$$

$$\text{CM (\%)} = \sum_{i-0}^{n} \left(\frac{\text{Mi}}{\text{Ni}}\right) \times 100$$

Where i is the day when a molt is observed, Mi is the number of molts on the day i, Ni is the number of shrimp in each treatment, and n is the number of days.

At the end of the experiment, shrimp from each treatment were boiled and shell color intensity was visually distinguished at 2 levels i.e. light orange color (1) and more intense orange color (2) for FS, or by pairwise comparisons for ranking all the boiled shrimp of SS from 1 (more shrimp with lighter orange) to 4 (more shrimp with darker orange).

Statistical analysis

Data were analyzed for significant differences using one-way ANOVA and Tukey post-hoc tests, provided in Minitab 18.0. Comparisons of means were carried out at 5% significance level ($p < 0.05$).

RESULTS AND DISCUSSION

Environmental parameters

During 28 days of the FS study period, average temperature (°C) was 27.18 ± 0.47 (ranging 26.3–28.3), pH was 8.18 ± 0.09 (ranging 7.9–8.3) and DO content (mg/L) was 6.17 ± 0.26 (ranging 5.39–7.67). Total ammonia nitrogen and nitrite were low during the experiment, with TAN (mg/L) of approximately 0.05 and NO_2^--N (mg/L) of 0.16 ± 0.16 (ranging 0.05–1). All water quality indices were regarded as suitable for shrimp culture in comparison to the optimal water quality provided by FAO (1978).

For SS, water quality parameters were recorded with average temperature (°C) of 27.81 ± 1.26 (ranging 25.1–33.3), pH of 8.09 ± 0.25 (ranging 7.0–9.3). TAN (mg/L) was in the range of 0.40 ± 0.28, and NO_2^--N (mg/L) fluctuated around 0.60 ± 0.40. These environmental factors are in the suitable range for normal development of whiteleg shrimp.

Growth performance of small juvenile shrimp fed with various formulae at 5‰ and 15‰ salinities

The changes in growth performance of *L. vannamei* juveniles cultured at different salinities and with different supplementations were given in Table 1 for the FS. At 5‰ salinity, the best growth performance was observed in treatment with formula 1, followed by formula 2 ($p > 0.05$), and significantly differed from formulae 3 and 4 ($p < 0.05$). In terms of WG and DWG, formulae 2, 3 and 4 showed no significant differences from the control 1. At 15‰ salinity, shrimp fed with formula 1 also showed the best growth performance in terms of FW, WG and DWG which were significantly different from shrimp fed with formulae 2, 3, 4 and normal feed (control 2) ($p < 0.05$). Overall, there were no significant differences among shrimp cultured at two salinities particularly in WG and DWG (control 1 versus control 2), however, supplementing formula 1 (5 mL/kg feed) in the diets drastically improved the body gain of FS shrimp juveniles with IW of approximately 1.57 g/individual.

Previous studies confirmed the necessary of supplementation of minerals e.g. magnesium, calcium and phosphorus in *L. vannamei* culture for good survival and growth (Velasco *et al.*, 1998, Kanazawa *et al.*, 1984), particularly at low salinity level of 2‰ (Cheng *et al.*, 2006). This study aligned with previous findings on mineral supplementation supporting shrimp growth (compared to the control treatments) with the best results at low salinity. Additionally, exceeding amount of some elements e.g. Fe, K and Se did not positively improve shrimp development in the experimental conditions.

Table 1. Weight gain parameters of first stage whiteleg shrimp juveniles supplemented with different formulae for 28 days at 5 mL/kg feed and reared at 5‰ and 15‰ salinities.

Formula-Salinity	**Mean IW:** 1.57 ± 0.12 g	FW (g)	WG (g)	DWG (g/day)
Formula 1-5‰	Dose: 5 mL/kg feed	7.84 ± 1.77[ab]	6.47 ± 1.77[a]	0.23 ± 0.06[a]
Formula 2-5‰	Dose: 5 mL/kg feed	7.26 ± 2.22[abc]	5.77 ± 2.22[ab]	0.21 ± 0.08[ab]
Formula 3-5‰	Dose: 5 mL/kg feed	6.79 ± 2.14[c]	5.18 ± 2.16[b]	0.18 ± 0.08[b]
Formula 4-5‰	Dose: 5 mL/kg feed	7.08 ± 2.14[c]	5.58 ± 2.15[b]	0.20 ± 0.08[b]
Control 1-5‰	Normal feed	7.28 ± 1.76[abc]	5.56 ± 1.76[b]	0.20 ± 0.06[b]
Formula 1-15‰	Dose: 5 mL/kg feed	7.93 ± 2.08[a]	6.37 ± 2.09[a]	0.23 ± 0.07[a]
Formula 2-15‰	Dose: 5 mL/kg feed	7.15 ± 1.85[c]	5.53 ± 1.85[b]	0.20 ± 0.07[b]
Formula 3-15‰	Dose: 5 mL/kg feed	7.23 ± 1.79[bc]	5.62 ± 1.78[b]	0.20 ± 0.06[b]
Formula 4-15‰	Dose: 5 mL/kg feed	7.15 ± 1.86[c]	5.53 ± 1.91[b]	0.20 ± 0.07[b]
Control 2-15‰	Normal feed	7.08 + 1.80[c]	5.53 ± 1.81[b]	0.20 ± 0.06[b]

Growth performance of big juvenile shrimp fed with various formulae and doses at a low salinity

Formulae 1 and 2 showed better results at low salinity of 5‰ in the FS test. They were chosen for SS investigation on bigger shrimp of IW ~9.8 g/individual. The results are presented in Table 2 with various growth indices i.e. FW, WG, DWG and DLG. There were variations in the length of experimental shrimp, but no significance among treatments. For comparing WG/DWG of shrimp fed with the two formulae in this SS test, treatments of formula 1 showed better growth than those of formula 2 or normal feed. These results aligned with those of FS shrimp tested with the two formulae at the same salinity. For the controls 3 and 4 without treated feed, rearing SS shrimp at 15‰ showed an enhanced growth but insignificantly in comparison to at 5‰.

For dose variations, a double dose of formula either 1 or 2 did not significantly improve SS shrimp growth. Interestingly, the lower dose (5 mL/kg feed) of formula 1 showed the highest growth performance among the treatments, which was also better than rearing shrimp at 15‰ (control 4). This may be explained that the balance of minerals in rearing shrimp should be a key factor, and over additions e.g. composition and/or quantity are not beneficial for shrimp growth and may introduce inhibitory effect on the growth. From the results of this test, using formula 1 at dose 5 mL/kg feed continuously for 28 days could be an optimal addition for SS shrimp of IW of ~9.8 g/individual.

Table 2. Weight and length gain parameters of second stage whiteleg shrimp juveniles supplemented with two formulae at various doses of 5 and 10 mL/kg feed for 28 days.

Formula-Salinity	**Mean IW:** 9.8 ± 1.2 g	FW (g)	WG (g)	DWG (g/day)	DLG (cm/day)
Formula 1-5‰	Dose: 5 mL/kg feed	15.1 ± 1.1[x]	5.30 ± 1.04[x]	0.19 ± 0.04[x]	0.043 ± 0.023[x]
Formula 1-5‰	Dose: 10 mL/kg feed	14.4 ± 0.6[xy]	4.60 ± 0.60[xy]	0.16 ± 0.02[xy]	0.037 ± 0.006[x]
Formula 2-5‰	Dose: 5 mL/kg feed	13.7 ± 0.6[y]	3.93 ± 0.64[y]	0.14 ± 0.02[y]	0.033 ± 0.005[x]
Formula 2-5‰	Dose: 10 mL/kg feed	13.9 ± 0.2[xy]	4.07 ± 0.15[xy]	0.15 ± 0.01[xy]	0.030 ± 0.001[x]
Control 3-5‰	Normal feed	13.7 ± 0.4[y]	3.90 ± 0.36[y]	0.14 ± 0.01[y]	0.026 ± 0.006[x]
Control 4-15‰	Normal feed	14.5 ± 1.0[xy]	4.73 ± 1.00[xy]	0.17 ± 0.03[xy]	0.034 ± 0.010[x]

Mineral salts are important for growth and development of living organisms to regulate chemical/biochemical processes (Hays, 1985, Ozcan, 2004). In aquatic environments, they also support animal growth and local ecosystems e.g. stimulating natural feed abundance and stabilizing water parameters (Boyd, 1998). Phosphate can be absorbed by phytoplankton which is naturally present in culture pond, thus a right dose of supplements may help maintain the community and its nutrient demand/survival to avoid quick changes in environments. Previous studies confirmed a suitable level of mineral supplementation is critical in shrimp growth to avoid overdosing/insufficient inhibition effects, even under osmotic stress or alkaline environments (Cheng *et al.*, 2006, Roy, 2020, Alagarsamy, 2014, Gopalakrishnan *et al.*, 2011). Thus a balancing addition of minerals/trace elements may be an important criterion, which is aligned with the results observed in this study.

Specific growth rate, molting, feed conversion ratio, survival rate and color ranking of experimental shrimp

For SGR, smaller shrimp FS had higher growth rates (3–5 times) than bigger shrimp SS (Table 3). Among FS treatments, using formula 1 at both salinities significantly support shrimp growth in most of the cases. A similar result has been obtained for SS shrimp of the treatment of the same formula 1 at 5 mL/kg feed, whereas the remaining treatments showed no significant differences in SGR.

No significant differences on FCR (Table 3) were recorded on shrimp of FS treatments ($p > 0.05$), although the values varied from ~1.17 in formula 1 treatment at 5‰ salinity to ~1.73 in formula 3 treatment at the same salinity. These FCR values were quite consistent at ~1.4 for treatments at 15‰. Nevertheless, in fewer treatments of SS, FCRs were significantly better in formula 1 treatments (~1.1, at 5 and 10 mL/kg feed) whilst other treatments were with FCR ~1.4 at 5‰ salinity.

For CM, results of FS shrimp varied from min 112.22% to max 246.11% among treatments (Table 3, examples in Fig. 2), but there were no significant differences ($p > 0.05$). During SS, shrimp molted faster when being reared at 15‰ or with formula 1 supplementations significantly ($p < 0.05$), which indicates a faster growth of shrimp.

Figure 2. Examples of different sizes of shrimp shells collected after molting.

SR assessing any impacts of continuous applications of formulae showed either improved or impaired effects on shrimp (Table 3). At 5‰ salinity, FS shrimp showed the highest mean SR in the treatment applying formula 1 at 5 mL/kg feed, and SS shrimp had significant differences ($p < 0.05$) in formula 1 treatments (both 5 and 10 mL/kg feed doses). At 15‰ salinity, an increased SR was recorded for formulae 1 and 2 which showed no significant differences among treatments. However, these treatments at 15‰ were significantly different from those applied formulae 2 and 3 at 5‰ salinity. Overall, shrimp of 15‰ salinity treatments had higher SR than that of 5‰ salinity in most of the cases.

Dietary mineral supplementation for shrimp is required for growth and performance due to losses during molting. Calcium, phosphorus and magnesium are three of essential minerals that have been found to be related to problems of soft-shelling syndrome in shrimp (Piedad-Pascual, 1989, Roy, 2020). Phosphorus deficiency results in reduced growth while lack of magnesium brings about slow growth, poor survival and reduced feed efficiency. Previous observation of iron toxicity on *Penaeus japonicus* shrimp (Piedad-Pascual, 1989) may show similar data on whiteleg shrimp in this study.

Table 3. Specific growth rate, feed conversion ratio, cumulative molting rate and survival rate of shrimp at two stages tested with various formulae, salinities and doses.

Treatment		Dose	SGR (%/day)	FCR	CM (%)	SR (%)
FS shrimp	**Formula 1-5‰**	5 mL/kg	5.90 ± 0.98[a]	1.17 ± 0.07[a]	244.81 ± 83.33[a]	88.89 ± 1.92[abc]
	Formula 2-5‰	5 mL/kg	5.42 ± 1.45[bc]	1.56 ± 0.22[a]	208.50 ± 36.29[a]	75.00 ± 4.41[bc]
	Formula 3-5‰	5 mL/kg	4.86 ± 1.67[d]	1.72 ± 0.19[a]	220.00 ± 31.11[a]	71.67 ± 7.64[c]
	Formula 4-5‰	5 mL/kg	5.33 ± 1.37[bcd]	1.63 ± 0.37[a]	214.81 ± 41.07[a]	78.33 ± 6.01[abc]
	Control 1-5‰	None	5.21 ± 1.15[bcd]	1.64 ± 0.21[a]	188.52 ± 38.18[a]	83.89 ± 13.37[abc]
	Formula 1-15‰	5 mL/kg	5.65 ± 1.32[ab]	1.39 ± 0.15[a]	167.04 ± 53.40[a]	94.45 ± 2.55[a]
	Formula 2-15‰	5 mL/kg	5.14 ± 1.20[cd]	1.37 ± 0.19[a]	151.85 ± 29.61[a]	93.89 ± 5.09[a]
	Formula 3-15‰	5 mL/kg	5.23 ± 1.06[bcd]	1.35 ± 0.12[a]	155.93 ± 31.35[a]	92.78 ± 4.20[ab]
	Formula 4-15‰	5 mL/kg	5.18 ± 1.24[cd]	1.40 ± 0.38[a]	195.00 ± 44.27[a]	92.78 ± 2.55[ab]
	Control 2-15‰	None	5.32 ± 1.20[bcd]	1.49 ± 0.17[a]	195.93 ± 3.57[a]	91.66 ± 5.77[ab]
SS shrimp	**Formula 1-5‰**	5 mL/kg	1.54 ± 0.24[x]	1.11 ± 0.09[x]	215.48 ± 4.03[x]	91.11 ± 1.92[x]
	Formula 1-5‰	10 mL/kg	1.37 ± 0.15[xy]	1.16 ± 0.02[xy]	214.00 ± 12.55[x]	90.00 ± 3.33[x]
	Formula 2-5‰	5 mL/kg	1.20 ± 0.16[y]	1.39 ± 0.13[z]	166.18 ± 15.89[y]	77.78 ± 5.09[y]
	Formula 2-5‰	10 mL/kg	1.24 ± 0.03[xy]	1.31 ± 0.09[yz]	160.72 ± 6.28[y]	78.89 ± 5.09[y]
	Control 3-5‰	None	1.20 ± 0.09[y]	1.38 ± 0.12[z]	158.64 ± 8.90[y]	76.67 ± 8.82[y]
	Control 4-15‰	None	1.40 ± 0.26[xy]	1.25 ± 0.11[xyz]	204.84 ± 9.25[x]	84.44 ± 1.92[xy]

In general, when comparing shrimp cultured at 5‰ and 15‰ salinities, growth performance and SR of shrimp were similar or higher across all the treatments conducted at salinity of 15‰ compared to the same treatments conducted at salinity of 5‰. The results in this study were in agreement with the findings of Li *et al.* (2007) and Jannathulla *et al.* (2017) which indicated that *L. vannamei* reared between 10‰ and 30‰ had better growth performance compared to lower salinity. The lower performance of *L. vannamei* at 5‰ may attribute to the fact that the animals have to spend more energy to compensate the cost for osmoregulation at low salinity (Li *et al.*, 2007).

For color intensity of shrimp boiled at the end of the test (Fig. 3), rearing shrimp at 15‰ gave a higher color intensity of shrimp with shell on than at 5‰ salinity. For FS, addition of various formulae enhanced the color of shrimp compared with the controls accordingly; among those, formula 1 showed its higher ranking. For SS, supplementing formulae 1 and 2 at 5 mL/kg feed and 5‰ salinity gave a color ranking at least as similar as the 15‰ salinity control 4. The continuous addition at 10 mL/kg feed with any formulae did not improve the shrimp color. A previous study applying a low concentration of copper (present in all formulae in this study) on the diet of whiteleg shrimp showed a significantly redder color of shrimp than that of the controls (Sakthivel *et al*., 2014). From this study, we added excessive amounts of minerals (including Fe, K and Se) that did not contribute in color enhancement. We are mindful that experimental shrimp were kept mostly in less sunlight (e.g. indoor and with cover) and clear water conditions, thus shrimp color was generally impacted.

	Treatment	Dose	Code	Color ranking
FS shrimp	**Formula 1-5‰**	5 mL/kg	a/b	1.19 ± 0.39[b]
	Formula 2-5‰	5 mL/kg	a/b	1.16 ± 0.36[b]
	Formula 3-5‰	5 mL/kg	a/b	1.12 ± 0.33[b]
	Formula 4-5‰	5 mL/kg	a/b	1.14 ± 0.35[b]
	Control 1-5‰	None	a/b	1.12 ± 0.33[b]
	Formula 1-15‰	5 mL/kg	a/b	1.35 ± 0.48[a]
	Formula 2-15‰	5 mL/kg	a/b	1.21 ± 0.41[ab]
	Formula 3-15‰	5 mL/kg	a/b	1.22 ± 0.42[ab]
	Formula 4-15‰	5 mL/kg	a/b	1.35 ± 0.48[a]
	Control 2-15‰	None	a/b	1.21 ± 0.41[b]
SS shrimp	**Formula 1-5‰**	5 mL/kg	A	Ranking 4
	Formula 1-5‰	10 mL/kg	B	Ranking 1
	Formula 2-5‰	5 mL/kg	C	Ranking 3
	Formula 2-5‰	10 mL/kg	D	Ranking 2
	Control 3-5‰	None	F	Ranking 2
	Control 4-15‰	None	E	Ranking 3

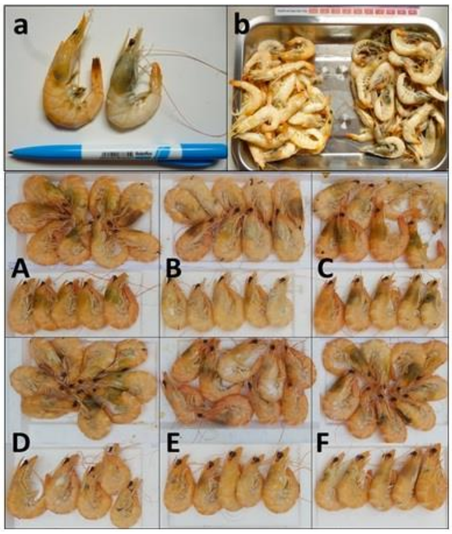

Figure 3. Color intensity of boiled shrimp after 28-day feeding supplementation of formulae

CONCLUSION

Even though the present study was conducted under a less favorable condition e.g. indoor, shrimp survival rate was high (up to ~95%) in suitable water parameters. This attributes to the overall quality of rearing conditions that were maintained during the experimental duration of 28 days. Treatments with formulae 2, 3 and 4 did not statistically improve any growth indices of whiteleg shrimp juveniles. The best product which showed significant and stable effectiveness on growth performance, feed utilization and color of shrimp at both 5‰ and 15‰ salinities was formula 1 (manufactured as Calciphos by Virbac) at both stages of juveniles. Cumulative molting rate was also significantly higher in formula 1 treatments at both doses i.e. 5 and 10 mL/kg feed, particularly for shrimp reared at low salinity 5‰. It has been concluded that formula 1 can introduce an ionic balance in shrimp farming, which supplementation in the feed is highly recommended for shrimp farming in low salinity area, or during periods of heavy raining that could induce major modification of the ionic profile of the water.

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