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Effect of silica supplement on growth performance and health condition of juvenile shrimp

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SILICA+ is activated quartz which improves the animal's digestive system by stimulating enzymatic exchange. In swine, Silica+ has a positive effect on growth performance by promoting not only growth but reducing ammonia emission in the environment. We also found that the product can increase the dissolved oxygen concentration in water and improve water quality as it decreases ammonia, nitrate and nitrite. The purpose of this study is to confirm whether the supplementation with this silica product has a positive effect on growth performance and whether it improves the health condition of Pacific white shrimp *Litopenaeus vannamei*.

A feeding trial was designed to determine if the inclusion of Silica+ (Ceresco Nutrition, Canada) in diets for juvenile shrimp could result in growth, feed efficiency and health conditions compared to shrimp fed a control diet.

Experimental details

The feeding trial on shrimp was conducted at the indoor facility of the Kidchakan Supamattaya Aquatic Animal Health Research Center, Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkhla University, Hat Yai, Thailand. Fibreglass tanks of 235-L (water volume 180-L) were used for rearing the shrimp. These were part of a continuously aerated system. The water temperature in the aquaria was 26-27°C, pH ranged from 7.2 to 7.8, and dissolved oxygen level was no less than 6.0 mg/l. Mean values for total ammonia and nitrite were 0.01 and 0.02 mg/l, respectively.

Shrimp post larvae (PL13) were obtained from Nattapon Hatchery, Songkhla, Thailand and maintained on a commercial feed in the experimental rearing units for 3 months before the start of feeding trials. The average body weight of the shrimp at the start of the feeding trial was 2.17g. Thirty shrimp were randomly released into each of the 24 tanks (6 tanks per treatment) in the set-up.

Feeds and feeding regime

Experimental diets were formulated with fishmeal (FM) as the major protein source. Other feed ingredients included dehulled soybean, poultry meal, wheat flour, squid liver meal and corn meal. Diet 1 (T1) contained 15% FM whereas diet 2 (T2) was formulated to contain equal amounts of FM and nutrients as in T1 but 0.02% silica supplementation was added. Diet 3 (T3) was formulated to contain 7.5% FM while diet 4 (T4) was formulated to contain the same amount of FM as T3 but with 0.02% silica supplementation. Feed composition is presented in Table 1. All diets contained nutrients according to the requirement of the shrimp. They provided 39% crude protein and 6% lipid (Table 2).

The diets were made into sinking pellets (pellet diameter: 2 mm) using a Hobart mixer (Model A200T, USA), oven-dried at 60°C and stored at -20°C. Details of diet preparation have been previously described in our former study (Phromkunthong et al., 2013). Shrimp were hand fed four times per day at 08:00, 12:00, 16:00 and 20:00, to satiety. The apparent intake of the experimental feeds was recorded every two weeks during the 8-week trial by visual inspection of the aquaria.

Performance

The bulk weights of shrimp were recorded from each tank at the start of the feeding period and every fortnight in order to estimate the weight gain. The initial body composition was determined from a minced sample obtained from 30 shrimp. At the end of the study, three shrimp were collected from each replicate of the six treatment tanks in order to analyse body composition, as described in our previous study (Kiron et al. 2012).

Table 1. Formulation of the experimental diets

Ingredients	Experimental diets			
	T1 (control)	T2 Silicia+	T3 (control)	T4 Silicia+
	FM 15%	FM 15%+Si+0.02%	FM 7.5%	FM 7.5%+Si+0.02%
Fish meal (FM)	15	15	7.5	7.5
Soybean de-hulled	21	21	29.5	29.5
Poultry meal	18	18	19.5	19.5
Wheat flour	3	3	3	3
Squid liver meal	2	2	2	2
Corn meal	5	5	5	5
Fish oil	0.9	0.9	1	1
Lecithin	1	1	1.3	1.3
Choline chloride	0.1	0.1	0.1	0.1
Vitamin & mineral premix (ROVIMIX 2050)	0.2	0.2	0.2	0.2
Inositol	0.04	0.04	0.04	0.04
Mono-ammonium phosphate	1.73	1.73	1.98	1.98
BHT	0.02	0.02	0.02	0.02
Calcium carbonate	0	0	0.82	0.82
Cassava flour	32.01	31.99	28.04	28.02
Silica (Si)	0	0.02	0	0.02
Total	100	100	100	100
Cost (THB/kg)	20.44	20.61	20.04	20.21

[1]Vitamin & Mineral premix (ROVIMIX®2050, DSM Nutritional Products) deliver the following in unit kg-1 diet: vitamin A 7,000 IU; Cholecalciferol (D3) 3,000 IU; Tocopherol (E) 1,500 mg; Menadione sodium bisulfite (K3) 30 mg; Thiamine (B1) 25 mg; Riboflavin (B2) 20 mg; Pyridoxine (B6) 25 mg; Cobalamin (B12) 0.02 mg; Niacin 100 mg; Calcium pantothenate 80 mg; Ascorbic acid (C) 200 mg; Biotin 1 mg; Folic acid 10 mg; Copper 25 mg; iron 30 mg; Zinc 100 mg; Manganese 30 mg; Cobalt 0.2 mg; Iodine 1 mg; Selenium 0.35 mg, Silica (Ceresco Nutrition, Canada)
Exchange rate: USD 1=THB32.5 on 31 March 2015

Table 2. Proximate composition of experimental diets[1](% dry matter basis)

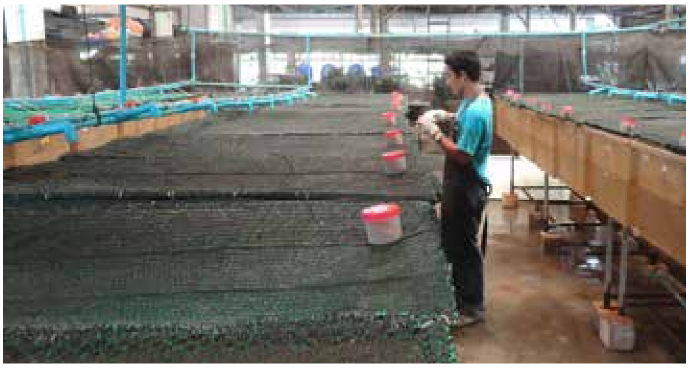
Diets	Moisture	Protein	Fat	Ash
T1 (control)	5.41±0.20	38.75±0.11	6.18±0.11	11.05±0.22
T2	5.31±0.03	39.72±0.15	6.15±0.10	10.84±0.10
T3 (control)	5.35±0.11	38.30±0.39	6.21±0.06	11.09±0.58
T4	5.24±0.14	39.72±0.14	6.20±0.12	10.86±0.39

[1]Analyses of 3 batches of feed given as mean ± SD

The performance parameters for the 8-week feeding period are presented in Table 4. Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) were presented.

Enzyme analyses

The activity of phenoloxidase (PO) and superoxide dismutase (SOD) was determined. The activity of total protease in the intestine was also analysed. Details of all measured parameters are indicated below.



Experimental tanks

Phenoloxidase (PO) activity

Haemolymph samples were individually drawn into microtubes containing cacodylate buffer (CAC, pH 7.4) at a dilution of 1:3 which were then immediately immersed in liquid nitrogen. Samples were homogenised with ice and centrifuged at 12,000 rpm for 15 min at 4˚C. Supernatant was collected into new microtubes for PO activity test.

Superoxide dismutase (SOD) activity

SOD activity was measured using a commercially available kit (Cayman Chem, USA) by the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine.

Total protease activity

Shrimp intestine was individually extracted based on the method of Vega-Villasante and co-worker (1995) with a slight modification. The protease activity assay was determined using casein as a substrate.

Statistical analysis

In this trial, FM*Si denotes interaction between FM levels (15% or 7.5%) and Si (with or without Si supplementation). If the statistical testing if P<0.05 in the table there are significant differences among the two parameters). Mean values are reported with 3 standard deviation of the mean (SD). After confirming normality and homogeneity of variance, the data were analysed by two-way ANOVA (SPSS, version 11.5), using fishmeal and Silica+ concentrations as the two factors. Where two-way ANOVA showed a significant interaction between the two factors, one-way ANOVA was used to identify significantly different means using Tukey multiple comparison. Differences were considered significant at P<0.05.

Results

There were significant differences in terms of average body weight from week 2 onwards as higher inclusion of FM (15%) gave higher growth compared to the lower FM level (7.5% FM) (P<0.05) (Figure 1). The inclusion of silica provided higher average body weight than that of un-supplemented groups in both the FM levels from week 4 onwards.

In addition, growth performance in terms of weight gain (WG), specific growth rate (SGR) and average daily growth (ADG) had the same trend as compared to average body weight (P<0.05) (Table 4).

Feed conversion ratio (FCR) of shrimp fed on diets incorporated with silica was better compared to un-supplemented diets in both FM levels (P<0.05) (Table 4). The average mortality for all the treatment groups was low (less than 10%), and there were non-significant differences between the treatment groups in survival (Table 4). The feed intake was insignificantly affected by the dietary silica supplementation (P>0.05) (Table 4).

Table 3. Average body weight of shrimp fed on the experimental diets for 8 weeks[1]

Experimental diets	Average body weight (g)				
	week 0	week 2	week 4	week 6	week 8
T1 (control)	2.17±0.00^{ax}	3.15±0.11^{bx}	4.23±0.19^{bx}	5.22±0.23^{bx}	6.79±0.34^{bx}
T2	2.17±0.00^{ax}	3.19±0.11^{bx}	4.39±0.05^{by}	5.52±0.32^{by}	7.29±0.25^{by}
T3 (control)	2.17±0.00^{ax}	2.89±0.08^{ax}	3.64±0.11^{ax}	4.66±0.21^{ax}	6.16±0.57^{ax}
T4	2.17±0.00^{ax}	2.99±0.09^{ax}	3.86±0.05^{ay}	4.97±0.45^{ay}	6.52±0.40^{ay}
FM level	0.329	0.000	0.000	0.000	0.000
Silica level	0.329	0.087	0.001	0.028	0.016
FM*Silica	0.329	0.470	0.561	0.964	0.726

[1]Mean ± SD from each of the 6 replicate tanks.
FM level ^{a,b} , Silica level ^{x,y}

Table 5. Protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) of shrimp fed on the experimental feeds for 8 weeks[1]

Experimental feeds	PER	ANPU
T1 (control)	1.17 ±0.05^{bx}	22.61±2.49^{bx}
T2	1.35 ±0.10^{by}	25.91± 3.02^{by}
T3 (control)	1.03 ±0.15^{ax}	18.80±2.26^{ax}
T4	1.09 ±0.08^{ay}	20.19±1.14^{ay}
FM level	0.000	0.000
Silica level	0.009	0.023
FM*Silica	0.194	0.328

[1]Mean ± SD from each of the 6 replicate tanks
FM level a,b , Silica level ^{x,y}
PER = wet weight gain (g)/protein intake (g)
ANPU (%) = 100 [(protein content (g) of fish at end of experiment protein content (g) of fish at start of experiment)]/fed protein, dry (g)

Table 4. Survival rate, weight gain (WG), specific growth rate (SGR), average daily growth (ADG), average feed intake and feed conversion ratio (FCR) of shrimp fed on the experimental feeds for 8 weeks[1]

Experimental feeds	Survival rate (%)	WG (%)	SGR (%/day)	ADG (g/day)	Average feed intake(g/shrimp)	FCR
T1 (control)	96.11±3.90^{ax}	212.76±15.76^{bx}	2.0±30.09^{bx}	0.08±0.01^{bx}	10.14±0.47^{ax}	2.24±0.12^{by}
T2	96.67±4.22^{ax}	235.71±11.39^{by}	2.16±0.06^{by}	0.09±0.00^{by}	9.61±0.64^{ax}	1.91±0.19^{bx}
T3 (control)	94.44±3.44^{ax}	183.02±25.94^{ax}	1.85±0.16^{ax}	0.07±0.01^{ax}	10.06±0.36^{ax}	2.66±0.41^{ay}
T4	92.78±3.90^{ax}	200.42±18.61^{ay}	1.96±0.11^{ay}	0.08±0.01^{ay}	10.01±0.35^{ax}	2.41±0.15^{ax}
FM level	0.094	0.000	0.000	0.000	0.401	0.000
Silica level	0.729	0.016	0.013	0.017	0.149	0.007
FM*Silica	0.491	0.719	0.853	0.739	0.217	0.724

[1]Mean ± SD from each of the 6 replicate tanks
FM level ^{a,b} , Silica level ^{x,y}
WG: weight gain, SGR: specific growth rate, ADG: average daily growth, FCR: feed conversion ratio were calculated using the following formulae:
SGR (% day^{-1}) = 100 [Ln (mean final body weight, g) – Ln (mean initial body weight, g)]/feeding days
FCR = dry feed intake (g)/[final biomass (g) initial biomass (g) + biomass of the dead fish (g)] PER = wet weight gain (g)/protein intake (g)
ANPU (%) = 100 [(protein content (g) of fish at end of experiment protein content (g) of fish at start of experiment)]/fed protein, dry (g)

Protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) gave the same trend as indicated for FCR. Shrimp fed on the diets containing silica with different FM levels had a higher PER and ANPU compared to unsupplemented diets (P<0.05) (Table 5). The supplementation of Silica+ seems to increase lipid content , especially in the diet formulated to contain 7.5% FM (<P 0.05) (Table 5).

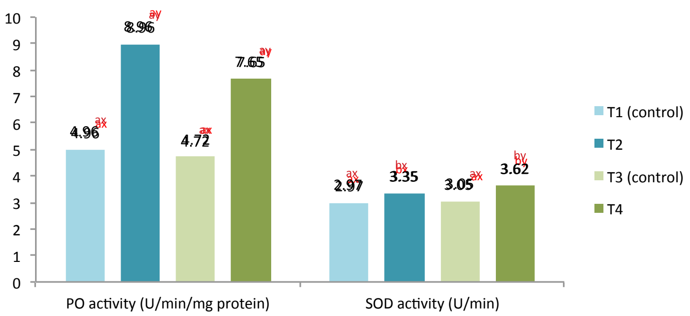
The supplementation of Silica+ in the shrimp diet formula significantly increased phenoloxidase activity (PO) and superoxide dismutase activity (SOD)(P<0.05). Furthermore, total protease activity in the intestine of shrimp fed diets supplemented with silica was higher when compared to that of the un-supplemented groups (P<0.05) (Figure 2).

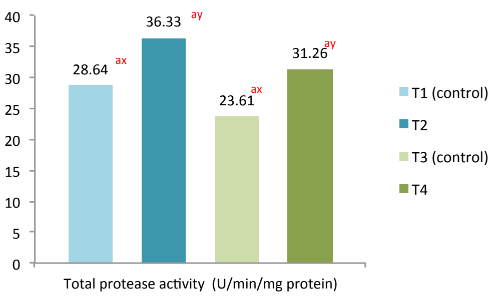
Table 6. Proximate composition of whole body shrimp fed on the experimental feeds for 8 weeks1 (% on dry matter basis)

Experimental feeds	Dry matter	Protein	Fat	Ash
T1 (control)	23.44±1.43^{ax}	73.76±0.85^{c}	3.28±0.45^{a}	12.77±0.39^{ax}
T2	23.99±1.26^{ax}	72.45±1.57^{b}	3.38±0.23^{a}	12.39±0.70^{ax}
T3 (control)	23.63±0.52^{ax}	70.27±0.40^{a}	3.07±0.14^{a}	12.57±0.21^{ax}
T4	23.63±0.74^{ax}	71.13±0.52^{a}	4.05±0.62^{b}	12.36±0.66^{ax}
FM level	0.847	0.000	0.178	0.603
Silica level	0.531	0.579	0.004	0.189
FM*Silica	0.536	0.011	0.015	0.708

Values (%) are given as mean ± SD; n = 5 shrimp from each of the 6 replicate tanks
FM level ^{a,b,c} · Silica level ^{x,y}

Figure 2. Blood parameters (phenol oxidase activity, PO; superoxide dismutase activity, SOD and total protease activity in the intestine of shrimps which conducted after the termination of growth study. Values are given as mean ± SD; n = 20 shrimp from each of the experimental feeds. FM level ^{a,b} , Silica level ^{x,y}





Conclusion

The results from this present study showed that the supplementation of Silica+ in shrimp diet enhanced growth performance and feed utilisation (FCR, PER and ANPU). Similar to other trace elements, silica may play an important role as cofactor in many biochemical processes in the cells of shrimp. It promoted immune system functions in shrimp as PO and SOD increased. These two endogenous antioxidants are crucial for the control of reactive oxygen species (ROS) production and the prevention of oxidative damage of cells (Lesser, 2006) and PO and SOD were increased when marine invertebrates are naturally exposed to stress conditions (Abele and Puntarulo, 2004; Guerriero et al., 2002; Martínez-Alvarez et al., 2005; Pannunzio and Storey, 1998). In our study, shrimp were not subjected to stress, but PO and SOD significantly increased as Silica+ had positive effects in this regard.

The elevation of the total protease activity in the gut of shrimp receiving diets supplemented with Silica+ suggests that this product has a positive influence on protein digestion.

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