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APPENDICES

APPENDIX

A: Chemical reagents

1. Cacodylate (CAC buffer); pH 7.0 and kept at 4 °C.

Sodium cacodylate	2.14 g.
$CaCl_2.2H_2O$	0.74 g.
$MgCl_2.6H_2O$	10.16 g.

The solid chemicals were dissolved in distilled water, adjusted to pH 7.0 and adjusted to volume 1,000 ml in volume metric flak. This solution was sterilized by autoclave for 15 min. at 121 °C, 15 pounds pressure and kept at 4 °C in a dark bottle.

2. Anticoagulant solution (AC-1); pH 7.0, kept at 4 °C.

NaCl	26.3 g.
Trisodium citrate	8.82 g.
Citric acid	5.46 g.
Na EDTA	3.72 g.

The chemical materials were dissolved in distilled water, adjusted to pH 7.0 and adjusted to volume 1,000 ml in volume metric flak. This solution was sterilized by autoclave for 15 min. at 121 °C, 15 pounds pressure and kept at 4 °C. Mix the solution with sterilized 0.1 M glucose, previously used.

3. 0.1% Trypsin; pH 7.0±0.2, kept at 4 °C.

Trypsin	0.1	g.
CAC buffer	100	ml.

The trypsin was dissolved in CAC buffer, adjusted to pH 7.0 and adjusted to volume 100 ml. in volume metric flak. This solution was sterilized by filtration through a 0.22 μm. pore diameter of membrane filter. This fresh solution was freshly prepared, before used, and kept in a dark bottle.

4. 0.3% L-DOPA; pH 7.0±0.2, kept at 4 °C.

L-3,4-dihydroxyphenylalanine	0.3	g.
CAC buffer	100	ml.

The L-3,4-dihydroxyphenylalanine was dissolved in CAC buffer, adjusted to pH 7.0 and adjusted to volume 100 ml in volume metric flak. This solution was sterilized by filtration through a 0.22 μm. pore diameter of membrane filter. This fresh solution was freshly prepared, before used, and kept in a dark bottle.

5. 5.0% L-cysteine; pH 7.0±0.2, kept at 4 °C.

L-cystein	5.0	g.
Lobster haemolymph medium	100	ml.

The L-cystein was dissolved in lobster haemolymph medium (LHM), adjusted to pH 7.0 and adjusted to volume 100 ml in volume metric flak. This solution was sterilized by filtration through a 0.22 μm. pore diameter of membrane filter. This fresh solution was freshly prepared, before used, and kept in a dark bottle.

6. 0.3% Eosin

Eosin Y	0.3	g
95% Ethyl alcohol	100	ml.
Glacial acetic acid	0.5	ml.

The chemical materials were dissolved in 95% ethyl alcohol and adjusted to volume 100 ml in volume metric flak.

7. Divison fixation (ml/L.)

Glacial acetic acid	115 ml
95% Ethyl alcohol	330 ml
39% Formaldehyde	220 ml
Distilled water	335 ml

The chemical reagents were mixed in distilled water and adjusted to volume 1,000 ml in volume metric flak.

8. Hematoxylin reagent compound of:

Hematoxylin crystals	2.0	g.
Sodium iodate	0.4	g.
Potassium aluminium sulfate	100	g.
Citric acid	2.0	g.
Chloral hydrate	100	g.
Distilled water	1,000	ml.

The chemical materials were dissolved in distilled water and adjusted to volume 1,000 ml in volume metric flak.

APPENDIX

B: Media

1. Agar media

1.1 Tryptic soy agar (TSA); pH 7.3±0.2

Peptone from casein	17.0	g.
Peptone from soymeal	3.0	g.
D (+) glucose	2.5	g.
Sodium chloride	5.0	g.
Di-potassium hydrogen phosphate	2.5	g.
Agar powder	15	g.

Dispensed solid media in distilled water until dissolved and sterilized by autoclave for 21 min. at 121 °C, 15 pounds pressure.

1.2 Thiosulfate citrate bile salt sucrose agar (TCBSA); pH 8.6±0.2

Yeast extract	5.0	g.
Proteose peptone No.3	10.0	g.
Sodium citrate	10.0	g.
Sodiumthiosulfate	10.0	g.
Oxgall	8.0	g.
Saccharose	20.0	g.
Sodium chloride	10.0	g.
Ferric citrate	1.0	g.
Bromthymol blue	0.04	g.
Agar powder	15.0	g

Dispensed solid media in distilled water until dissolved and sterilized by autoclave for 21 min. at 121 °C, 15 pounds pressure.

1.3 Mueller Hinton agar (MHA); pH 7.3±0.2

Beef extract powder	2.0	g.
Casein hydrolysate	17.5	g.
Soluble starch	1.5	g.
Agar powder	13.0	g.

Dispensed solid media in distilled water until dissolved and sterilized by autoclave for 21 min. at 121 °C, 15 pounds pressure.

2. Both media

2.1 Mueller Hinton broth (MHB); pH 7.3±0.2

Beef extract powder	2.0	g.
Casein hydrolysate	17.5	g.
Soluble starch	1.5	g.

Dispensed solid media in distilled water until dissolved and sterilized by autoclave for 21 min. at 121 °C, 15 pounds pressure.

2.2 Lobster haemolymph medium (LHM); pH 7.6 ±0.2 and kept at 4 °C.

MEM essential amino acid with L-glutamine (2X)	500	ml.
11% NaCl (w/v)	100	ml.
11% $CaCl_2.2H_2O$ (w/v)	100	ml.
Hepes buffer	2.38	g/L.
Steriled water	200	ml.
Salt mixture reagent compound of:	100	ml.
- KCl	0.4	g.
- $NaH_2PO_4.2H_2O$	0.05	g.

All solid materials were dissolved in distilled water and mixed with all chemical solutions adjusted to pH 7.6 with 7.5% $NaHCO_3$ and adjusted to volume 1,000 ml in volume metric flak. This medium was sterilized by filtration through a 0.45 μm. pore diameter of membrane filter.

APPENDIX

C: Table

Table 13. Feeding of *Penaeus monodon* shrimp number 100,000 pieces have density 25 pieces per a square meter.

Week	Weight gain (g/pcs.)	Growth rate (g/pcs/day)	Survival rate (%)	Feeding rate per shrimp weight (%)	Frequency of feedind rate per day (a time)
1	PL15-0.10	0.03	95	100	3
2	0.50	0.06	92	20	3
3	1.00	0.07	90	10	3
4	1.80	0.11	88	10	3
5	2.80	0.14	86	9.0	3
6	3.90	0.16	84	8.0	4
7	5.50	0.23	83	7.0	4
8	7.60	0.30	82	7.0	4
9	9.80	0.31	81	6.0	4
10	12.00	0.31	80	5.0	4
11	14.30	0.33	79	5.0	4
12	17.10	0.40	78	4.0	4
13	20.00	0.41	77	4.0	5
14	23.30	0.47	76	4.0	5
15	26.70	0.49	75	4.0	5
16	30.60	0.56	74	3.5	5

Table 14. Effect of polysaccharide gel (PG) from durian fruit-hulls on growth performances of *Penaeus monodon* juvenile in the trial rearing were evaluated after every 4 weeks for 8 weeks and 12 weeks feeding period, using four groups of shrimp (100 shrimps/group) in each treatments. Values are mean ± SD. Control = 0.0% PG. ns = not significant. a, b, c = significantly different between groups, ($P<0.05$).

Subject	Shrimp number (n)	Growth performance after feeding with shrimp diets with different concentration of PG. (mean ± SD)			
		Control	0.5% PG	1.0% PG	2.0% PG
1. body weight (gram)					
0 day	4 group	0.36 ± 0.00^{ns}	0.36 ± 0.00^{ns}	0.36 ± 0.00^{ns}	0.36 ± 0.00^{ns}
4 weeks	4 group	1.77 ± 0.21^{ns}	1.89 ± 0.18^{ns}	2.04 ± 0.10^{ns}	2.03 ± 0.21^{ns}
8 weeks	4 group	5.90 ± 0.37^{b}	5.65 ± 0.19^{b}	6.06 ± 0.16^{ab}	6.55 ± 0.48^{a}
2. survival rate (%)					
4 weeks	4 group	92.00 ± 6.53^{ns}	92.00 ± 7.30^{ns}	94.00 ± 2.31^{ns}	89.00 ± 6.00^{ns}
8 weeks	4 group	57.00 ± 5.03^{c}	75.00 ± 8.87^{a}	69.00 ± 6.00^{ab}	63.00 ± 6.83^{cb}
3. body length (mm.)					
0 day	30 pcs.	3.84 ± 0.10^{ns}	3.84 ± 0.10^{ns}	3.84 ± 0.10^{ns}	3.84 ± 0.10^{ns}
4 weeks	15 pcs.	65.88 ± 4.74^{ns}	64.73 ± 5.27^{ns}	67.93 ± 4.82^{ns}	67.94 ± 4.68^{ns}
8 weeks	30 pcs.	93.60 ± 6.96^{ns}	93.44 ± 7.74^{ns}	94.39 ± 7.24^{ns}	97.27 ± 8.48^{ns}
4. FCR					
4 weeks	4 group	1.88 ± 0.51^{ns}	1.70 ± 0.29^{ns}	1.47 ± 0.08^{ns}	1.60 ± 0.21^{ns}
8 weeks	4 group	2.56 ± 0.17^{b}	1.98 ± 0.30^{a}	2.02 ± 0.24^{a}	2.09 ± 0.36^{a}
5. Biomass					
4 weeks	4 group	40.82 ± 6.85^{ns}	43.48 ± 6.01^{ns}	47.93 ± 2.20^{ns}	45.15 ± 4.86^{ns}
8 weeks	4 group	83.79 ± 5.03^{b}	105.91 ± 12.45^{a}	104.74 ± 11.60^{a}	103.44 ± 15.93^{a}

Table 15. Water quality during the feeding period in the trial rearing was evaluated at every week.

Environmental category	Value of water quality (minimum-maximum)
pH	7.6-8.2
Salinity (ppt)	16-21
Hardness (ppm)	2,100-3,250
Temperature (°C)	29.5-33.0
Nitrite, NO_2 (ppm)	0.0-0.2
Total alkalinity (ppm)	100-160
Dissolved oxygen, DO (ppm)	3.5-6.0
Total ammonia nitrogen, TAN (ppm)	0.0-0.2

Table 16. The survival rate of black tiger shrimp (*Penaeus monodon*) on challenge test with WSSV, 10^6 (1:100 dilution) by cohabitation method after 4 weeks feeding period, using three groups of the rearing shrimp (30 shrimp/group) in each treatments. Values are mean ± SD. Control = 0.0% PG.

Date	The survival rate of black tiger shrimp with different concentration of PG. (%)			
	Control	0.5% PG	1.0% PG	2.0% PG
0	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
1	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
2	97 ± 0.58	100 ± 0.00	100 ± 0.00	97 ± 0.58
3	97 ± 0.58	100 ± 0.00	100 ± 0.00	97 ± 0.58
4	93 ± 0.58	100 ± 0.00	100 ± 0.00	97 ± 0.58
5	83 ± 1.53	93 ± 1.15	97 ± 0.58	97 ± 0.58
6	70 ± 1.00	83 ± 0.58	97 ± 0.58	87 ± 0.58
7	53 ± 1.53	57 ± 0.58	97 ± 0.58	77 ± 1.15
8	20 ± 1.73	37 ± 0.58	93 ± 1.15	60 ± 1.73
9	13 ± 1.53	17 ± 1.53	90 ± 1.00	47 ± 3.06
10	3 ± 0.58	10 ± 1.73	80 ± 1.00	20 ± 3.46
11	0 ± 0.00	0 ± 0.00	57 ± 1.53	17 ± 2.89
12	0 ± 0.00	0 ± 0.00	27 ± 0.58	10 ± 1.73
13	0 ± 0.00	0 ± 0.00	7 ± 0.58	3 ± 0.58
14	0 ± 0.00	0 ± 0.00	3 ± 0.58	0 ± 0.00

Table 17. The survival rate of black tiger shrimp (*Penaeus monodon*) on challenge test with WSSV, 10^6 (1:100 dilution) by cohabitation method after 8 weeks feeding period, using three groups of the rearing shrimp (24 shrimp/group) in each treatments. Values are mean ± SD. Control = 0.0% PG.

Date	The survival rate of black tiger shrimp with different concentration of PG. (%)			
	Control	0.5% PG	1.0% PG	2.0% PG
0	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
1	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
2	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
3	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
4	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
5	100 ± 0.00	100 ± 0.00	83 ± 1.15	96 ± 0.58
6	100 ± 0.00	96 ± 0.58	58 ± 3.06	83 ± 2.31
7	100 ± 0.00	96 ± 0.58	54 ± 3.21	75 ± 3.46
8	88 ± 0.00	92 ± 1.15	42 ± 4.04	71 ± 4.04
9	79 ± 0.58	67 ± 2.08	33 ± 4.62	71 ± 4.04
10	54 ± 0.58	38 ± 2.65	25 ± 3.46	58 ± 4.16
11	42 ± 1.15	21 ± 2.89	13 ± 1.73	50 ± 3.61
12	21 ± 1.15	21 ± 2.89	8 ± 1.15	46 ± 3.51
13	4 ± 0.58	17 ± 2.31	4 ± 0.58	38 ± 3.61
14	0 ± 0.00	4 ± 0.58	4 ± 0.58	33 ± 3.79

Table 18. The survival rate of black tiger shrimp (*Penaeus monodon*) on challenge test with *Vibrio harveyi* 1526, 2.15×10^7 CFU/ml by immersion method after 8 weeks feeding period, using three groups of the rearing shrimp (24 shrimp/group) in each treatments. Values are mean ± SD. Control = 0.0% PG.

Date	The survival rate of black tiger shrimp In different concentration of PG. (%)			
	Control	0.5% PG	1.0% PG	2.0% PG
0	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
1	100 ± 1.00	67 ± 0.58	71 ± 0.58	88 ± 1.73
2	50 ± 1.00	67 ± 0.58	67 ± 0.58	75 ± 3.46
3	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
4	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
5	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
6	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
7	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
8	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
9	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
10	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
11	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
12	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
13	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46
14	50 ± 1.00	58 ± 0.58	67 ± 0.58	75 ± 3.46

Table 19. Water quality of black tiger shrimp (*Penaeus monodon*) on challenge test with WSSV, 10^6 (1:100 dilution) by cohabitation method after 4 weeks feeding period was evaluated at everyday.

Environmental category	Value of water quality (minimum-maximum)
pH	7.9-8.2
Salinity (ppt)	15
Nitrite, NO_2 (ppm)	0.0-0.5
Temperature (^oC)	27-28
Total ammonia nitrogen, TAN (ppm)	0.0-0.5

Table 20. Water quality of black tiger shrimp (*Penaeus monodon*) on challenge test with *Vibrio harveyi* 1526, $2.15x10^7$ CFU/ml by immersion method after 8 weeks feeding period was evaluated at everyday.

Environmental category	Value of water quality (minimum-maximum)
pH	7.9-8.2
Salinity (ppt)	15
Nitrite, NO_2 (ppm)	0.0-0.5
Temperature (^oC)	29-30
Total ammonia nitrogen, TAN (ppm)	0.0-0.5

Table 21. Water quality of black tiger shrimp (*Penaeus monodon*) on challenge test with WSSV, 10^6 (1:100 dilution) by cohabitation method after 8 weeks feeding period was evaluated at everyday.

Environmental category	Value of water quality (minimum-maximum)
pH	7.9-8.2
Salinity (ppt)	15
Nitrite, NO_2 (ppm)	0.0-0.5
Temperature (^oC)	27-28
Total ammonia nitrogen, TAN (ppm)	0.0-0.5

Table 22. Antimicrobial activity of PG inhibits growth of bacteria *Vibrio harveyi* 1526 by agar diffusion metod. nz = no inhibition zone.

Concentration of PG (%)	Diameter of inhibition zone (mm.)					
5.0	19.0	18.8	20.1	22.9	19.6	22.2
2.5	15.1	15.6	15.9	17.5	18.6	16.1
1.25	11.4	11.7	11.6	12.7	12.5	13.0
0.63	10.8	11.4	10.8	10.6	10.9	9.7
0.32	9.2	9.6	9.1	8.2	8.5	8.7
NSS	nz	Nz	nz	Nz	nz	nz

Table 23. Total haemocyte count (THC) of *Penaeus monodon* juvenile shrimp were counted using haemacytometer after 8 weeks feeding period, using 12 shrimps/group in each treatments. Values are mean ± SD. Control = 0.0% PG.

No. sample (piece)	Total haemocyte count (THC) (10^7 cells/ml)			
	Control	0.5% PG	1.0% PG	2.0% PG
1	1.75 ± 0.25	1.29 ± 0.19	2.13 ± 0.13	2.33 ± 0.19
2	1.21 ± 0.26	1.38 ± 0.13	1.04 ± 0.19	1.58 ± 0.26
3	1.54 ± 0.19	1.58 ± 0.38	1.08 ± 0.19	0.83 ± 0.07
4	0.58 ± 0.07	1.00 ± 0.22	1.00 ± 0.25	2.33 ± 0.19
5	1.58 ± 0.07	1.25 ± 0.13	1.79 ± 0.19	1.54 ± 0.19
6	1.33 ± 0.19	0.79 ± 0.07	1.54 ± 0.07	1.46 ± 0.07
7	1.17 ± 0.07	2.04 ± 0.19	1.21 ± 0.26	2.46 ± 0.19
8	0.75 ± 0.13	0.88 ± 0.22	2.04 ± 0.07	1.96 ± 0.07
9	0.54 ± 0.07	1.17 ± 0.19	2.08 ± 0.19	1.04 ± 0.07
10	0.54 ± 0.07	1.79 ± 0.26	1.92 ± 0.07	1.00 ± 0.22
11	1.63 ± 0.13	0.58 ± 0.07	1.63 ± 0.22	2.21 ± 0.19
12	1.29 ± 0.14	0.67 ± 0.07	1.25 ± 0.25	1.46 ± 0.07
Average	1.16 ± 0.45	1.20 ± 0.45	1.56 ± 0.43	1.68 ± 0.57

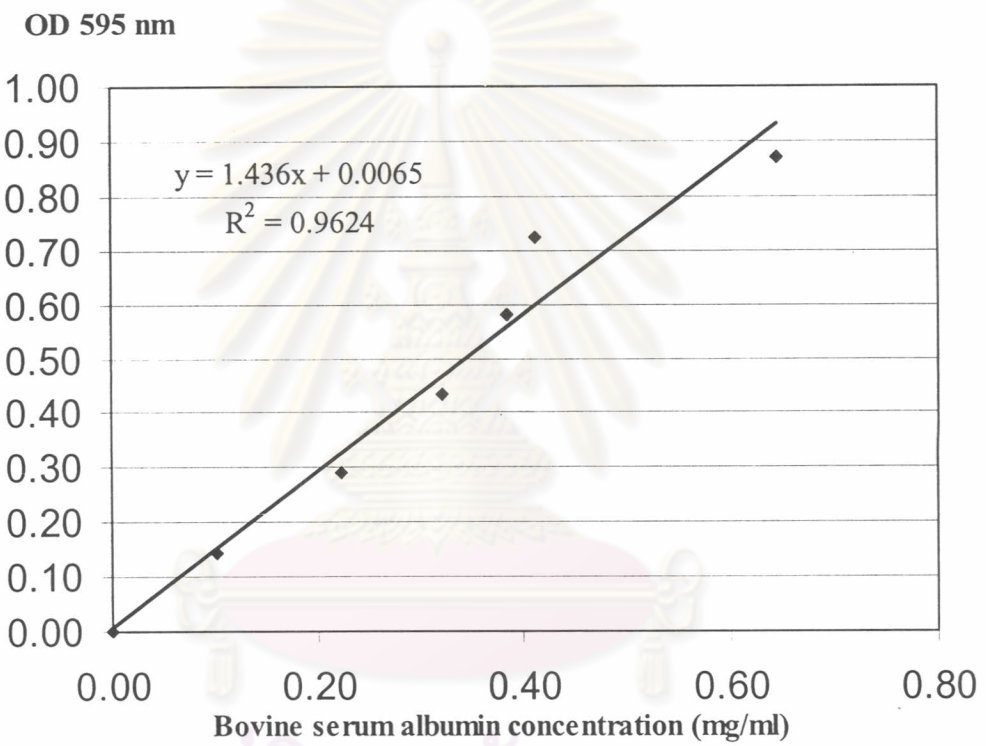
Table 24. Total phenoloxidase activity of *Penaeus monodon* juvenile shrimp were measured in the haemolymph after 8 weeks feeding period, using 12 shrimps/group in each treatments. Control = 0.0% PG.

No. sample (piece)	The phenoloxidase activity (PO) (units/min./mg protein)			
	Control	0.5% PG	1.0% PG	2.0% PG
1	620.00	812.50	659.57	777.78
2	547.17	207.79	898.55	1125.00
3	538.46	928.57	740.74	571.43
4	571.43	702.13	1232.56	956.52
5	584.62	857.14	1290.32	1235.29
6	595.74	844.83	1078.43	1083.33
7	596.49	980.77	1319.15	1081.08
8	509.43	613.64	1035.71	1127.66
9	756.10	637.93	698.11	511.11
10	839.29	651.16	615.38	743.59
11	741.94	513.16	714.29	948.72
12	411.76	722.22	518.52	558.14
Average	609.37±117.71	705.99±208.97	900.11±281.85	893.30±252.58

APPENDIX

D: Figure

Figure 31. Standard curve of bovine serum albumin protein by Bradford protein assay for phenoloxidase activity test.



APPENDIX

E: Statistics

The biomass of shrimp feeding period for 4 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	40.8225	6.85032	3.42516	29.9221	51.7229	30.66	45.00
2	4	43.4750	6.00947	3.00473	33.9126	53.0374	36.54	51.12
3	4	47.9250	2.20158	1.10079	44.4218	51.4282	45.77	50.16
4	4	45.1525	4.85873	2.42936	37.4212	52.8838	39.79	51.36
Total	16	44.3438	5.42228	1.35557	41.4544	47.2331	30.66	51.36

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	106.533	3	35.511	1.274	.328
Within Groups	334.484	12	27.874		
Total	441.018	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-2.6525	3.73321	.491	-10.7865	5.4815
	3	-7.1025	3.73321	.081	-15.2365	1.0315
	4	-4.3300	3.73321	.269	-12.4640	3.8040
2	1	2.6525	3.73321	.491	-5.4815	10.7865
	3	-4.4500	3.73321	.256	-12.5840	3.6840
	4	-1.6775	3.73321	.661	-9.8115	6.4565
3	1	7.1025	3.73321	.081	-1.0315	15.2365
	2	4.4500	3.73321	.256	-3.6840	12.5840
	4	2.7725	3.73321	.472	-5.3615	10.9065
4	1	4.3300	3.73321	.269	-3.8040	12.4640
	2	1.6775	3.73321	.661	-6.4565	9.8115
	3	-2.7725	3.73321	.472	-10.9065	5.3615

The biomass of shrimp feeding period for 8 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	83.7925	5.0275	2.5138	75.7926	91.7924	78.82	90.72
2	4	105.9075	12.4472	6.2236	86.1013	125.7137	88.32	116.97
3	4	104.7350	11.6018	5.8009	86.2739	123.1961	93.44	117.80
4	4	103.4350	15.9292	7.9646	78.0881	128.7819	82.18	120.78
Total	16	99.4675	14.2087	3.5522	91.8962	107.0388	78.82	120.78

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1322.667	3	440.889	3.102	.067
Within Groups	1705.651	12	142.138		
Total	3028.319	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-22.1150(*)	8.4302	.022	-40.4829	-3.7471
	3	-20.9425(*)	8.4302	.029	-39.3104	-2.5746
	4	-19.6425(*)	8.4302	.038	-38.0104	-1.2746
2	1	22.1150(*)	8.4302	.022	3.7471	40.4829
	3	1.1725	8.4302	.892	-17.1954	19.5404
	4	2.4725	8.4302	.774	-15.8954	20.8404
3	1	20.9425(*)	8.4302	.029	2.5746	39.3104
	2	-1.1725	8.4302	.892	-19.5404	17.1954
	4	1.3000	8.4302	.880	-17.0679	19.6679
4	1	19.6425(*)	8.4302	.038	1.2746	38.0104
	2	-2.4725	8.4302	.774	-20.8404	15.8954
	3	-1.3000	8.4302	.880	-19.6679	17.0679

* The mean difference is significant at the 0.05 level.

The feed conversion ratio (FCR) of shrimp feeding period for 4 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	1.8750	.5055	.2528	1.0706	2.6794	1.59	2.63
2	4	1.6950	.2931	.1465	1.2286	2.1614	1.36	2.07
3	4	1.4700	8.165E-02	4.082E-02	1.3401	1.5999	1.39	1.55
4	4	1.6000	.2099	.1050	1.2660	1.9340	1.35	1.85
Total	16	1.6600	.3189	7.972E-02	1.4901	1.8299	1.35	2.63

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.349	3	.116	1.185	.357
Within Groups	1.177	12	9.805E-02		
Total	1.525	15			

Post Hoc tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.1800	.2214	.432	-.3024	.6624
	3	.4050	.2214	.092	-7.7424E-02	.8874
	4	.2750	.2214	.238	-.2074	.7574
2	1	-.1800	.2214	.432	-.6624	.3024
	3	.2250	.2214	.330	-.2574	.7074
	4	9.500E-02	.2214	.675	-.3874	.5774
3	1	-.4050	.2214	.092	-.8874	7.742E-02
	2	-.2250	.2214	.330	-.7074	.2574
	4	-.1300	.2214	.568	-.6124	.3524
4	1	-.2750	.2214	.238	-.7574	.2074
	2	-9.5000E-02	.2214	.675	-.5774	.3874
	3	.1300	.2214	.568	-.3524	.6124

The feed conversion ratio (FCR) of shrimp feeding period for 8 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	2.5625	.1666	8.330E-02	2.2974	2.8276	2.34	2.74
2	4	1.9825	.3017	.1509	1.5024	2.4626	1.71	2.41
3	4	2.0175	.2403	.1202	1.6351	2.3999	1.76	2.26
4	4	2.0875	.3630	.1815	1.5099	2.6651	1.77	2.61
Total	16	2.1625	.3465	8.663E-02	1.9779	2.3471	1.71	2.74

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.876	3	.292	3.789	.040
Within Groups	.925	12	7.708E-02		
Total	1.801	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.5800(*)	.1963	.012	.1523	1.0077
	3	.5450(*)	.1963	.017	.1173	.9727
	4	.4750(*)	.1963	.032	4.728E-02	.9027
2	1	-.5800(*)	.1963	.012	-1.0077	-.1523
	3	-3.5000E-02	.1963	.861	-.4627	.3927
	4	-.1050	.1963	.603	-.5327	.3227
3	1	-.5450(*)	.1963	.017	-.9727	-.1173
	2	3.500E-02	.1963	.861	-.3927	.4627
	4	-7.0000E-02	.1963	.728	-.4977	.3577
4	1	-.4750(*)	.1963	.032	-.9027	-4.7278E-02
	2	.1050	.1963	.603	-.3227	.5327
	3	7.000E-02	.1963	.728	-.3577	.4977

* The mean difference is significant at the 0.05 level.

The total length of shrimp feeding period for 4 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean (mm)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	12	65.8750	4.73654	1.36732	62.8655	68.8845	55.60	75.20
2.00	12	64.7250	5.27087	1.52157	61.3761	68.0739	55.70	71.00
3.00	12	67.9250	4.82138	1.39181	64.8616	70.9884	62.00	79.70
4.00	12	67.9417	4.67711	1.35017	64.9700	70.9134	58.90	72.60
Total	48	66.6167	4.92468	.71082	65.1867	68.0466	55.60	79.70

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	91.150	3	30.383	1.275	.295
Within Groups	1048.717	44	23.834		
Total	1139.867	47			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	1.1500	1.99309	.567	-2.8668	5.1668
	3	-2.0500	1.99309	.309	-6.0668	1.9668
	4	-2.0667	1.99309	.305	-6.0835	1.9501
2	1	-1.1500	1.99309	.567	-5.1668	2.8668
	3	-3.2000	1.99309	.116	-7.2168	.8168
	4	-3.2167	1.99309	.114	-7.2335	.8001
3	1	2.0500	1.99309	.309	-1.9668	6.0668
	2	3.2000	1.99309	.116	-.8168	7.2168
	4	-.0167	1.99309	.993	-4.0335	4.0001
4	1	2.0667	1.99309	.305	-1.9501	6.0835
	2	3.2167	1.99309	.114	-.8001	7.2335
	3	.0167	1.99309	.993	-4.0001	4.0335

The total length of shrimp feeding period for 8 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean (mm)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	30	93.6033	6.96437	1.27151	91.0028	96.2039	76.60	104.20
2	30	93.4400	7.74203	1.41349	90.5491	96.3309	80.00	113.70
3	30	94.3900	7.24247	1.32229	91.6856	97.0944	81.60	108.70
4	30	97.2667	8.47944	1.54813	94.1004	100.4329	79.40	114.00
Total	120	94.6750	7.68892	.70190	93.2852	96.0648	76.60	114.00

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	284.150	3	94.717	1.627	.187
Within Groups	6751.075	116	58.199		
Total	7035.225	119			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.1633	1.96975	.934	-3.7380	4.0647
	3	-.7867	1.96975	.690	-4.6880	3.1147
	4	-3.6633	1.96975	.065	-7.5647	.2380
2	1	-.1633	1.96975	.934	-4.0647	3.7380
	3	-.9500	1.96975	.631	-4.8513	2.9513
	4	-3.8267	1.96975	.054	-7.7280	.0747
3	1	.7867	1.96975	.690	-3.1147	4.6880
	2	.9500	1.96975	.631	-2.9513	4.8513
	4	-2.8767	1.96975	.147	-6.7780	1.0247
4	1	3.6633	1.96975	.065	-.2380	7.5647
	2	3.8267	1.96975	.054	-.0747	7.7280
	3	2.8767	1.96975	.147	-1.0247	6.7780

The survival of shrimp feeding period for 4 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	23.0000	1.63299	.81650	20.4015	25.5985	21.00	25.00
2	4	23.0000	1.82574	.91287	20.0948	25.9052	21.00	25.00
3	4	23.5000	.57735	.28868	22.5813	24.4187	23.00	24.00
4	4	22.2500	1.50000	.75000	19.8632	24.6368	21.00	24.00
Total	16	22.9375	1.38894	.34724	22.1974	23.6776	21.00	25.00

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.188	3	1.063	.495	.692
Within Groups	25.750	12	2.146		
Total	28.938	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.0000	1.03582	1.000	-2.2569	2.2569
	3	-.5000	1.03582	.638	-2.7569	1.7569
	4	.7500	1.03582	.483	-1.5069	3.0069
2	1	.0000	1.03582	1.000	-2.2569	2.2569
	3	-.5000	1.03582	.638	-2.7569	1.7569
	4	.7500	1.03582	.483	-1.5069	3.0069
3	1	.5000	1.03582	.638	-1.7569	2.7569
	2	.5000	1.03582	.638	-1.7569	2.7569
	4	1.2500	1.03582	.251	-1.0069	3.5069
4	1	-.7500	1.03582	.483	-3.0069	1.5069
	2	-.7500	1.03582	.483	-3.0069	1.5069
	3	-1.2500	1.03582	.251	-3.5069	1.0069

The survival of shrimp feeding period for 8 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	14.25	1.26	.63	12.25	16.25	13	16
2	4	18.75	2.22	1.11	15.22	22.28	16	21
3	4	17.25	1.50	.75	14.86	19.64	16	19
4	4	15.75	1.71	.85	13.03	18.47	14	18
Total	16	16.50	2.31	.58	15.27	17.73	13	21

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.000	3	15.000	5.143	.016
Within Groups	35.000	12	2.917		
Total	80.000	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-4.50(*)	1.21	.003	-7.13	-1.87
	3	-3.00(*)	1.21	.029	-5.63	-.37
	4	-1.50	1.21	.238	-4.13	1.13
2	1	4.50(*)	1.21	.003	1.87	7.13
	3	1.50	1.21	.238	-1.13	4.13
	4	3.00(*)	1.21	.029	.37	5.63
3	1	3.00(*)	1.21	.029	.37	5.63
	2	-1.50	1.21	.238	-4.13	1.13
	4	1.50	1.21	.238	-1.13	4.13
4	1	1.50	1.21	.238	-1.13	4.13
	2	-3.00(*)	1.21	.029	-5.63	-.37
	3	-1.50	1.21	.238	-4.13	1.13

* The mean difference is significant at the 0.05 level.

The body weight of shrimp feeding period for 4 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	1.7675	.21407	.10703	1.4269	2.1081	1.46	1.95
2	4	1.8875	.18062	.09031	1.6001	2.1749	1.74	2.13
3	4	2.0400	.09866	.04933	1.8830	2.1970	1.93	2.15
4	4	2.0325	.20759	.10379	1.7022	2.3628	1.73	2.19
Total	16	1.9319	.19951	.04988	1.8256	2.0382	1.46	2.19

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.203	3	.068	2.064	.159
Within Groups	.394	12	.033		
Total	.597	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2.00	-.1200	.12810	.367	-.3991	.1591
	3.00	-.2725	.12810	.055	-.5516	.0066
	4.00	-.2650	.12810	.061	-.5441	.0141
2	1.00	.1200	.12810	.367	-.1591	.3991
	3.00	-.1525	.12810	.257	-.4316	.1266
	4.00	-.1450	.12810	.280	-.4241	.1341
3	1.00	.2725	.12810	.055	-.0066	.5516
	2.00	.1525	.12810	.257	-.1266	.4316
	4.00	.0075	.12810	.954	-.2716	.2866
4	1.00	.2650	.12810	.061	-.0141	.5441
	2.00	.1450	.12810	.280	-.1341	.4241
	3.00	-.0075	.12810	.954	-.2866	.2716

The body weight of shrimp feeding period for 8 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	5.8975	.3689	.1845	5.3105	6.4845	5.63	6.43
2	4	5.6500	.1885	9.425E-02	5.3500	5.9500	5.52	5.93
3	4	6.0625	.1638	8.189E-02	5.8019	6.3231	5.84	6.20
4	4	6.5475	.4758	.2379	5.7903	7.3047	5.87	6.98
Total	16	6.0394	.4470	.1117	5.8012	6.2775	5.52	6.98

ANOVA

Analysis

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.722	3	.574	5.404	.014
Within Groups	1.275	12	.106		
Total	2.996	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.2475	.2305	.304	-.2546	.7496
	3	-.1650	.2305	.488	-.6671	.3371
	4	-.6500(*)	.2305	.015	-1.1521	-.1479
2	1	-.2475	.2305	.304	-.7496	.2546
	3	-.4125	.2305	.099	-.9146	8.962E-02
	4	-.8975(*)	.2305	.002	-1.3996	-.3954
3	1	.1650	.2305	.488	-.3371	.6671
	2	.4125	.2305	.099	-8.9618E-02	.9146
	4	-.4850	.2305	.057	-.9871	1.712E-02
4	1	.6500(*)	.2305	.015	.1479	1.1521
	2	.8975(*)	.2305	.002	.3954	1.3996
	3	.4850	.2305	.057	-1.7118E-02	.9871

* The mean difference is significant at the 0.05 level.

The total hemocyte count (THC) of shrimp feeding period for 8 weeks was statistical analyses by using one way ANOVA test in version 11.5.

One way

Descriptives

Analysis

group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	12	1.1592	.4484	.1294	.8743	1.4441	.54	1.75
2	12	1.2017	.4490	.1296	.9164	1.4870	.58	2.04
3	12	1.5592	.4313	.1245	1.2851	1.8332	1.00	2.13
4	12	1.6833	.5668	.1636	1.3232	2.0434	.83	2.46
Total	48	1.4008	.5146	7.427E-02	1.2514	1.5502	.54	2.46

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.435	3	.812	3.569	.021
Within Groups	10.009	44	.227		
Total	12.445	47			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-4.2500E-02	.1947	.828	-.4349	.3499
	3	-.4000(*)	.1947	.046	-.7924	-7.5789E-03
	4	-.5242(*)	.1947	.010	-.9166	-.1317
2	1	4.250E-02	.1947	.828	-.3499	.4349
	3	-.3575	.1947	.073	-.7499	3.492E-02
	4	-.4817(*)	.1947	.017	-.8741	-8.9246E-02
3	1	.4000(*)	.1947	.046	7.579E-03	.7924
	2	.3575	.1947	.073	-3.4921E-02	.7499
	4	-.1242	.1947	.527	-.5166	.2683
4	1	.5242(*)	.1947	.010	.1317	.9166
	2	.4817(*)	.1947	.017	8.925E-02	.8741
	3	.1242	.1947	.527	-.2683	.5166

* The mean difference is significant at the 0.05 level.

The phenoloxidase activity of shrimp feeding period for 8 weeks was statistical analyses by using one way ANOVA test in version 11.5.

Descriptives

Analysis

group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	12	609.3692	117.71273	33.98074	534.5781	684.1603	411.76	839.29
2	12	705.9867	208.96827	60.32394	573.2146	838.7588	207.79	980.77
3	12	900.1108	281.85145	81.36351	721.0310	1079.1907	518.52	1319.15
4	12	893.3042	252.58072	72.91377	732.8220	1053.7863	511.11	1235.29
Total	48	777.1927	250.54339	36.16282	704.4425	849.9429	207.79	1319.15

ANOVA

Analysis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	741909.333	3	247303.111	4.927	.005
Within Groups	2208374.144	44	50190.321		
Total	2950283.477	47			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Analysis

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-96.6175	91.46067	.297	-280.9444	87.7094
	3	-290.7417(*)	91.46067	.003	-475.0685	-106.4148
	4	-283.9350(*)	91.46067	.003	-468.2619	-99.6081
2	1	96.6175	91.46067	.297	-87.7094	280.9444
	3	-194.1242(*)	91.46067	.039	-378.4510	-9.7973
	4	-187.3175(*)	91.46067	.047	-371.6444	-2.9906
3	1	290.7417(*)	91.46067	.003	106.4148	475.0685
	2	194.1242(*)	91.46067	.039	9.7973	378.4510
	4	6.8067	91.46067	.941	-177.5202	191.1335
4	1	283.9350(*)	91.46067	.003	99.6081	468.2619
	2	187.3175(*)	91.46067	.047	2.9906	371.6444
	3	-6.8067	91.46067	.941	-191.1335	177.5202

* The mean difference is significant at the 0.05 level.

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