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Acute Toxicity of Ammonia and Nitrite to White Shrimp *Litopenaeus vannamei* Juveniles

Abstract

Two groups of juvenile white shrimp *Litopenaeus vannamei* with average body weight of 0.33 ± 0.11g and 0.15 ± 0.09g were exposed to different concentrations of ammonia and nitrite to determine the median lethal concentration (LC_{50}) by a static renewal method. The corresponding 24, 48, 72 and 96 h LC_{50} of ammonia were 3.70, 2.27, 1.83 and 1.65 mg/*l* for NH_3-N (un-ionized ammonia as nitrogen) and 71.51, 43.79, 35.30 and 31.80 mg/*l* for ammonia-N (un-ionized plus ionized ammonia as nitrogen), in 21‰ seawater at pH 7.9-8.3 and at 27℃. The 24, 48, 72 and 96 h LC_{50} of nitrite-N were 164.2, 47.9, 32.5 and 23.9 mg/*l*, respectively, in 21‰ seawater at pH 7.9-8.3 and at 27℃. The safe values of NH_3-N, ammonia-N and nitrite-N for white shrimp were 0.17, 3.18 and 2.4 mg/*l*, respectively.

Key words : Ammonia, Nitrite, White shrimp *Litopenaeus vannamei*, Acute toxicity

White shrimp *Litopenaeus vannamei* is the most widely cultured shrimp in the western hemisphere [1]. Rapid growth, good survival in high density culture, and disease tolerance make the shrimp as a good choice for the semi-intensive and intensive grow-out strategies employed in the USA[2,3].

Over the past twenty years, white spot syndrome virus (WSSV), monodon baculovirus (MBV), and pathogenic vibrio has devastated the tiger shrimp *Penaeus monodon* and Kuruma shrimp *Marsupenaeus japonicus* culture industry in Taiwan and other Asian countries[4-6]. White shrimp *L. vannamei* was then introduced into Taiwan in 1994, and commercially cultured in 1998 [7,8].

L. vannamei inhabits on wide range salinity from 1-2‰ to 40‰[9]. Although the optimal salinity for *L. vannamei* is not conclusive[10-13]. Huang[10] suggested that *L. vannamei* grew best at about 20‰.

In Taiwan, land suitable for aquaculture is scarce and expensive. Regulations governing the use of coastal land and water resources are also becoming more restrictive. These along with the increasing demand for shrimp products are bringing about the need to develop aquaculture technologies of white shrimp based on intensive culture.

One of the most important limiting factors in intensive culture systems is the build-up of toxic nitrogenous wastes, such as ammonia and urea[14]. In an intensive shrimp culture system, ammonia and nitrite increase exponentially over time in grow-out ponds[15,16]. High concentrations of ammonia may result in retardation of shrimp growth, and in extreme cases can cause mortality [17,18].

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Previous studies on the toxicity of ammonia and nitrite to shrimp have involved *P. monodon* [19-23], *M. japonicus*[18,24-26], *Metapenaeus ensis*[27-30], *Fenneropenaeus chinensis*[31-33] and *F. penicillatus*[34-36].

Toxicities of ammonia and nitrite on other species have been studied by other researchers [17,37-40]. However, little information is available on the toxicity of ammonia and nitrite to white shrimp [41,42].

This study tested the toxicity of ammonia and nitrite to *L. vannamei* juveniles.

Materials and Methods

Fifth substage postlarva (PL_5) of *L. vannamei* from a private nursery were shipped to the research laboratory at the Taiwan Fisheries Research Institute in Keelung. The shrimp were fed with artificial plankton and freshly hatched *Artemia nauplii.*

Seawater (33‰) pumped from Keelung coast adjacent to the Institute was filtered through a gravel and sand bed by air-lifting, and aerated for 3 days before use. Water temperature was maintained at 25-27℃ during the rearing period.

The salinity of this study was conducted at 20‰, and to keep the variation within ±1‰. The shrimp were acclimated to 20‰ seawater for 2 wk before the experiment. Two groups of shrimp, with an average body weight of 0.33±0.11g and 0.15±0.09g, an average total length of 3.31±0.37 cm and 2.61±0.49 cm, were used for the ammonia and nitrite tests, respectively.

Ammonia test solutions were prepared by dissolving 19.095g of ammonium chloride (Merck reagent grade) in 1*l* distilled water to make 5000 mg/*l* ammonia-N (un-ionized plus ionized ammonia as nitrogen), and then diluted to the desired nominal concentrations with 20‰ seawater. The desired concentrations of ammonia-N ranged, in 5 mg/*l* intervals, from 25 to 55 mg/*l*, 70 and 80 mg/*l*. The actual concentrations of ammonia-N were 25.26, 31.73, 35.20, 37.36, 43.88, 51.14, 55.71, 74.32 and 83.00 mg/*l* (1.31, 1.64, 1.82, 1.94, 2.27, 2.65, 2.89, 3.85 and 4.30 mg/*l* NH_3-N, un-ionized ammonia as nitrogen). The concentrations of NH_3-N were calculated according to the equations of Bower and Bidwell [43] with salinity of 21‰, pH 7.9-8.3 and water temperature 27℃.

Nitrite test solutions were prepared by dissolving 98.60 g of sodium nitrite (Merck reagent grade) in 1*l* distilled water to make 20000 mg/*l* nitrite-N (nitrite as nitrogen). The desired concentrations of nitrite-N were 10, 20, 25, 30, 50, 55, 60, 80, 130, 230, 260 and 300 mg/*l*. The actual concentrations of nitrite-N were 9.54, 19.43, 26.77, 29.72, 49.15, 51.53, 56.35, 85.99, 129.16, 232.83, 260.20 and 292.83mg/*l*.

A median lethal concentration (LC_{50}) study was conducted using a static renewal procedure. The shrimps were collected at random from the holding tanks and exposed to each test solution and a 0 mg/*l* control in duplicate tanks. Bioassay experiments to establish tolerance limits were conducted in 4 *l* test tanks containing 2 *l* of the test solution. Each tank was placed in a water bath (27℃) and contained 10 shrimps. All tanks were aerated continuously during the test. Each test solution was renewed every 24 h [44]. The shrimp were fed with a commercial shrimp diet (35% crude protein, Tairoun Products Co., LTD, Taipei, Taiwan) three times a day based on 6.0% of the body weight.

During the ammonia test experiment, water temperatures were maintained at 27℃, dissolved oxygen was 5.1-5.6 mg/*l*, salinity was 21‰, and pH ranged from 7.9-8.3. During the nitrite test experiment, water parameters were kept constant at 27℃, 5.2-5.8 mg/*l* DO, 21‰, 7.9-8.3 pH units.

Water samples from each tank were analyzed daily for ammonia and nitrite level. Actual total ammonia and nitrite concentrations were measured using the indophenol method[45] and azo-dye method [46].

Observations were made at 24 h intervals to 96 h for ammonia and nitrite tests. Death was assumed when shrimps were immobile and showed no response when touched with a glass rod. The dose response of test organisms, combined from duplicate tanks at each test concentration, was determined by plotting the probit of mortality transformed from percent mortality against log concentration[44]. The LC_{50} and 95% confidence limits were calculated with a computer program [47].

Results

Mortality of *L. vannamei* juveniles exposed to 4.30, 3.85, 2.89 and 2.65 mg/*l* un-ionized ammonia-N was 100% after 48, 48, 72 and 96 h, respectively (Table 1). However, shrimp exposed to 1.31 mg/*l* survived after 48 h. Mortality of the shrimps exposed to 292.83, 260.20 and 232.83 mg/*l* nitrite-N was 100% after 48, 48 and 72 h, respectively (Table 2). No shrimp died in the 0 mg/*l* control solution during 96 h of exposure.

The probit of mortality for *L. vannamei* juveniles exposed to ammonia or nitrite was linearly related to log un-ionized ammonia-N and log nitrite-N, respectively, at various exposure times (Tables 3, 4).

Table 1. Percent mortality of white shrimp *Litopenaeus vannamei* juveniles exposed to different concentrations of un-ionized ammonia-N and total ammonia-N after various time intervals.

Time elapsed (h)	*Un-ionized ammonia-N (mg/l) (Total ammonia-N, mg/l)*								
	1.31 (25.26)	*1.64 (31.73)*	*1.82 (35.20)*	*1.94 (37.36)*	*2.27 (43.88)*	*2.65 (51.14)*	*2.89 (55.71)*	*3.85 (74.32)*	*4.30 (83.00)*
	Mortality(%)								
24	0	5	25	20	25	25	35	45	65
48	0	5	30	20	50	55	70	100	100
72	10	20	50	50	80	90	100	100	100
96	15	35	60	90	95	100	100	100	100

Table 2. Percent mortality of white shrimp *Litopenaeus vannamei* juveniles exposed to different concentrations of nitrite-N after various time intervals.

Time elapsed (h)	*Nitrite-N (mg/l)*											
	9.54	*19.43*	*26.77*	*29.72*	*49.15*	*51.53*	*56.35*	*85.99*	*129.16*	*232.83*	*260.20*	*292.83*
	Mortality (%)											
24	10	25	10	20	20	10	15	20	5	50	75	80
48	10	45	30	45	45	35	55	40	35	80	100	100
72	10	55	45	55	70	65	55	75	75	100	100	100
96	15	60	80	75	75	90	70	90	90	100	100	100

The LC_{50} and its 95% confidence limits of ammonia-N, NH_3-N and nitrite-N are shown in Figs. 1 and 2. The LC_{50} of ammonia-N and nitrite-N on *L. vannamei* juveniles decreased with increase in exposure time. At 24, 48, 72 and 96 h, the LC_{50}s of NH_3-N (their 95% confidence limits are shown in parentheses) were 3.70 (2.81-4.89), 2.27 (2.11-2.44), 1.83 (1.71-1.96) and 1.65 (1.54-1.76) mg/*l*, and those of ammonia-N were 71.51 (54.22-94.31), 43.79 (40.68-47.14), 35.30 (32.97-37.80) and 31.80 (29.73-34.01) mg/*l*, respectively. The tolerance of juvenile *L. vannamei* to un-ionized ammonia-N decreased by 39, 51 and 55% after 48, 72 and 96 h of exposure, with respect to 24 h LC_{50} values. The LC_{50} of ammonia-N on *L. vannamei* juveniles sharply declined from 24 to 48 h and then stabilized to reach an asymptote.

The 24, 48, 72 and 96 h LC_{50} of nitrite-N (their 95% confidence limits are shown in parentheses) were 164.2 (117.4-229.8), 47.9 (33.7-67.8), 32.5 (24.0-43.9) and 23.9 (16.6-34.4) mg/*l*, respectively. The tolerance of juvenile to nitrite-N decreased by 71, 82 and 85% after 48, 72 and 96 h of exposure, with respect to 24 h LC_{50} values. The LC_{50} of nitrite-N on *L. vannamei* juveniles sharply declined from 24 to 48 h and then stabilized to reach an asymptote.

Table 3. Relationship between probit of mortality (Y) and log un-ionized ammonia-N concentration (X) at various exposure times on the juveniles of white shrimp *Litopenaeus vannamei*.

Time elapsed(h)	*Y = a + b X*	*N*	*r*[a]
24	*Y =-6.82+3.622X*	7	0.899
48	Y =-11.063+11.848X	6	0.906
72	Y =-10.518+12.295X	7	0.936
96	Y =-12.832+14.655X	6	0.911

[a] : Coefficient of correlation.

Table 4. Relationship between probit of mortality (Y) and log nitrite-N concentration (X) at various exposure times on the juveniles of white shrimp *Litopenaeus vannamei*.

Time elapsed(h)	*Y = a + b X*	*N*	*r*[a]
24	Y =-0.615+2.534X	6	0.924
48	Y =-1.737+2.514X	6	0.881
72	Y =-1.973+2.777X	7	0.951
96	Y =-1.440+2.708X	6	0.894

[a]: Abbreviation referring to the footnote of Table 3.

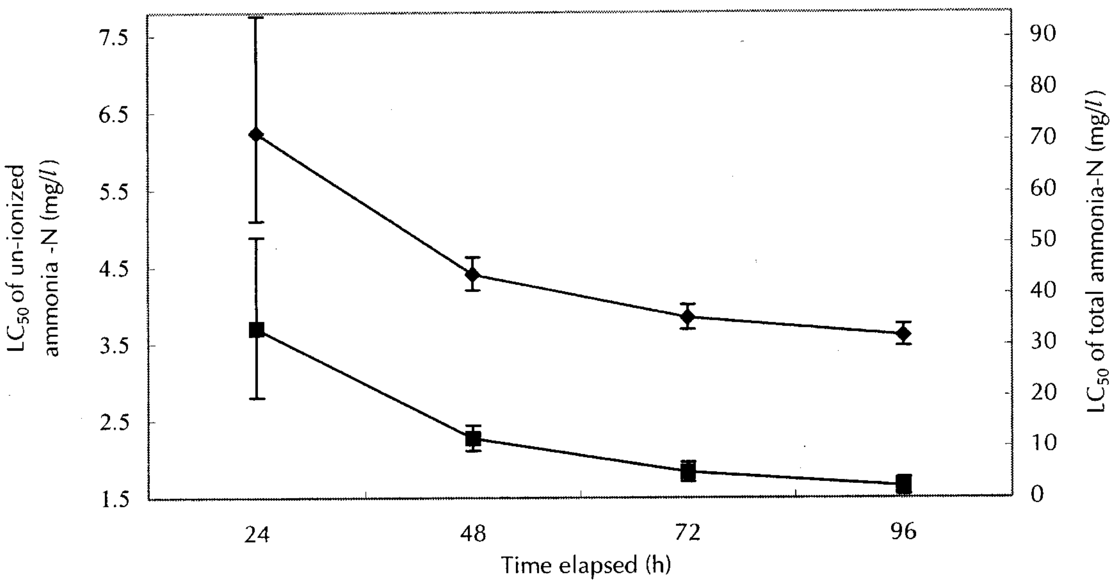

Fig. 1. LC_{50} (median lethal concentration) and their 95% confidence limits of total ammonia-N and un-ionized ammonia-N on the juveniles of white shrimp *Litopenaeus vannamei* (-■- un-ionized ammonia-N, -◆- total ammonia-N).

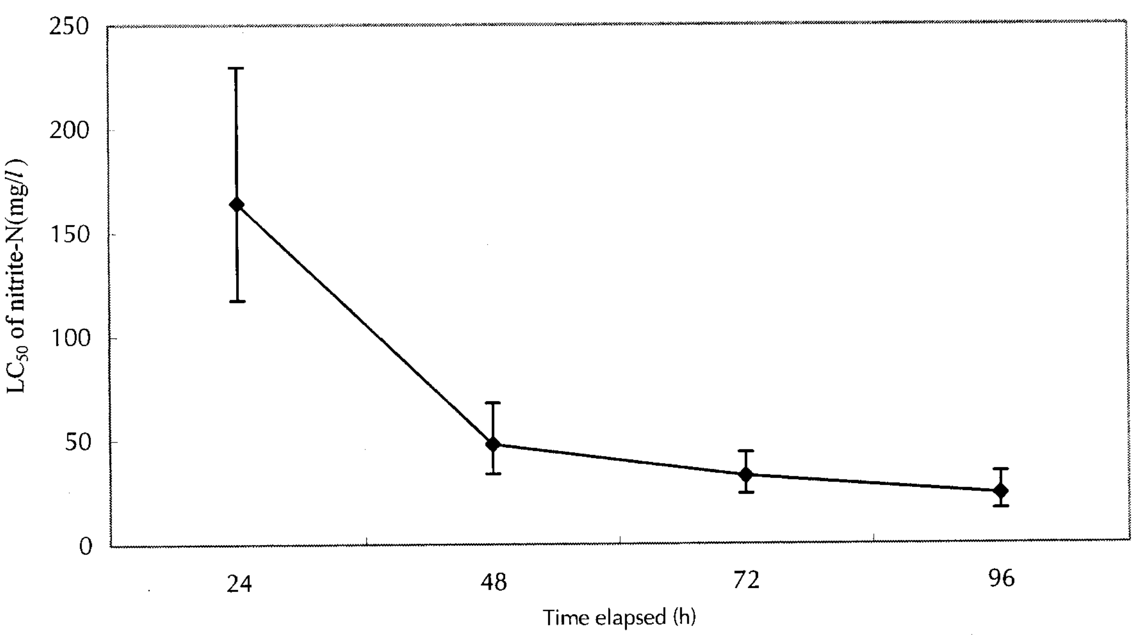

Fig. 2. LC_{50} and their 95% confidence limits of nitrite-N on the juveniles of white shrimp *Litopenaeus vannamei*.

Discussion

Toxic effects of ammonia on the juveniles of different species of penaeid shrimps are given in Table 5. The 24, 48, 72 and 96 h LC_{50} values for un-ionized ammonia-N in *L. vannamei* juvenile were lower than those reported for *M. japonicus* and *F. penicillatus* (Table 5)[18,26,36]. Therefore, this study suggests that *L. vannamei* juvenile is more sensitive to ammonia exposure than the juveniles of these two species. The 24, 48, 72 and 96 h LC_{50} values for un-ionized ammonia-N on *L. vannamei* juvenile were higher than those reported for *P. semisulcatus, Metapenaeus macleayi, P. monodon, M. ensis* and *Farfantepeneaus paulensis*[17,21-23,29,40], suggesting that *L. vannamei* juveniles are more tolerant than these five species of shrimp.

The 24, 48 and 96 h LC_{50} values for un-ionized ammonia-N on *L. vannamei* juveniles were similar to that reported for *F. chinensis* [31,33]. The 48 h LC_{50} value for un-ionized ammonia-N on *L. vannamei* (2.27 mg/*l*) was higher than the mean value (1.29 mg/*l* NH_3-N) reported by Wickins for seven species of penaeids[37].

Table 5. LC_{50} values of un-ionized ammonia-N (NH_3-N, mg/*l*) in several species of penaeid juveniles.

Species	Weight (g) or Length (cm)	pH	Salinity (‰)	Temp.(℃)	24 h	48 h	72 h	96 h	Reference
M. japonicus	0.025±0.003g, 1.34±0.05 cm	8.23	34	27	5.39	3.36	3.21	3.00	18
M. japonicus	10.4±1.1g	7.9-8.2	36±1	20	4.5	3.5	3.2	3.1	26
F. penicillatus	0.4-0.69g,	8.10	25	20	2.0	1.25	-	0.99	35
	3.58-4.75 cm	8.10	34	20	2.22	1.44	-	1.11	
F. penicillatus	0.17-0.50g, 3.36-4.53 cm	8.2	25	26	8.16	5.37	-	3.52	36
P. monodon	0.27±0.06g, 3.54±0.22 cm	7.70	20	26-28	2.68	1.73	1.35	1.29	22
P. monodon	4.87±1.4g, 9.1±0.8 cm	7.57	20	24.5	1.76	1.59	1.20	0.96	23
M. ensis	0.012±0.004g, 1.07±0.14 cm	7.7	25	25	2.19	1.76	1.50	0.87	29
F. paulensis	5.45±0.4 g	7.78	28	25±1	1.47	1.22	1.13	1.10	40
M. macleayi	0.9-4.8 g	-	34	25±1	-	1.66	-	1.39	21
P. semisulcatus	0.35-2.4 g	8.1	40.5	27	-	-	-	1.43	17
penaeid	0.5-1.5 g	8.0	28	28	-	1.29	-	-	37
F. chinensis	3.61±0.23 cm	8.12	20	25	3.31	2.70	-	2.46	33
F. chinensis	0.36±0.06 g, 3.96±0.18 cm	7.94	33	26	3.29	2.10	-	1.53	31
L. vannamei	2.2±0.24 cm	8.05	15	23	2.95	2.00	1.59	1.20	42
		8.05	25	23	2.93	2.16	1.91	1.57	
		8.05	35	23	2.78	2.18	1.82	1.60	
L.vannamei	0. 33±0.11 g, 3.31±0.37 cm	7.9-8.3	21	27	3.70	2.27	1.83	1.65	Present study

The 24, 48, 72 and 96 h LC_{50} values for un-ionized ammonia-N on *L. vannamei*[42] was similar to those of this work (Table 5). The size shrimp used (average total length 2.2 cm) was smaller than that of this study (3.31 cm). It is suggested that tolerance to ammonia of shrimp increase with age[23].

Toxic effects of nitrite on the juveniles of different species of penaeid shrimps are given in Table 6. The 24, 48, 72 and 96 h LC_{50} values for nitrite-N on *L. vannamei* juvenile were lower than those reported for *P. monodon* juvenile[22,23]. These results suggest that *L. vannamei* juvenile is more sensitive to nitrite than *P. monodon*.

Table 6. LC_{50} values of nitrite-N (NO_2-N, mg/*l*) in several species of penaeid juveniles.

Species	*Weight (g) or Length (cm)*	*pH*	*Salinity (‰)*	*Temp.(℃)*	*24 h*	*48 h*	*72 h*	*96 h*	*Reference*
P. monodon	0.27±0.06g, 3.54±0.22cm	7.7	20	26-28	215.85	185.53	88.54	54.76	22
P. monodon	4.87±1.4g, 9.1±0.8cm	7.57	20	24.5	217.64	192.66	185.31	171.09	23
F. chinensis	0.36±0.06g, 3.96±0.18cm	-	33	26	339	286	117	37.71	31
M. ensis	0.024±0.008g, 1.4±0.42cm	7.60	25	28	113	80.26	56.85	50.71	27
F. penicillatus	0.4-0.69g, 3.58-4.75cm	8.10 8.10	25 34	20 20	83.34 92.88	52.93 79.45	- -	38.52 40.86	35
penaeid	0.5-1.5g	8.0	28	28	-	170	-	-	37
L. vannamei	0.15±0.09g, 2.61±0.49cm	7.9-8.3	21	27	164.23	47.85	32.46	23.88	Present study

The 24, 48, 72 and 96 h LC_{50} values for nitrite-N on *F. chinensis* were also higher than these in *L. vannamei* [31], but the salinity of that experiment (33‰) was higher than that of the present study (21‰).

The 48 h LC_{50} of nitrite-N on seven penaeid shrimps tested combined together in 28‰ seawater was 170 mg/*l*[37], higher than that on *L. vannamei*. The 24 h LC_{50} (25‰ seawater) of nitrite on *M. ensis* juveniles were lower than that on *L. vannamei*. After 48 h *M. ensis* was more tolerant to nitrite than *L. vannamei*[27]. The toxicity of nitrite to fish is greatly affected by the salinity of the water in which the nitrite exposure occurred[48,49].

Based on the 96 h value LC_{50} and an empirical application factor of 0.1[50], the "safe level" for rearing *L. vannamei* was calculated to 3.18 mg/*l* ammonia-N, 0.17 mg/*l* NH_3-N, and 2.4 mg/*l* nitrite-N in 21 ‰ seawater at pH 7.9-8.3 and at a water temperature of 27℃.

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氨與亞硝酸對白蝦 *Litopenaeus vannamei* 稚蝦之急性毒研究

摘 要

白蝦的稚蝦 (平均體重分別爲 0.33±0.11 公克及 0.15±0.09 公克) 曝露在不同濃度的氨及亞硝酸中，以決定彼等對白蝦的稚蝦其 24、48、72 及 96 小時的半致死濃度（Median lethal concentration, LC_{50}）。非離子化氨-氮對白蝦的稚蝦之 24、48、72 及 96 小時的 LC_{50} 分別爲 3.70、2.27、1.83 及 1.65 mg/*l* (水溫 27℃、鹽度 21‰、pH 值 7.9-8.3)，而總氨-氮則分別爲 71.51、43.79、35.30 及 31.80 mg/*l*。亞硝酸-氮對白蝦的稚蝦之 24、48、72 及 96 小時的 LC_{50} 分別爲 164.2、47.9、32.5 及 23.9 mg/*l* (水溫、鹽度和 pH 值分別爲 27℃、21‰、7.9-8.3)。建議白蝦飼育期間，非離子化氨-氮、總氨-氮及亞硝酸-氮之濃度分別不可超過 0.17、3.18 及 2.4 mg/*l*。

關鍵詞：氨，亞硝酸，白蝦，急性毒

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