Effects of water exchange and reducing dietary vitamin and mineral supplementation on survival and growth of *Litopenaeus vannamei*

Lan-mei Wang[1,2], Addison L. Lawrence ⋆[2], Frank Castille[2], and Yun-long Zhao[1]

[1]Life Science College, East China Normal University, Shanghai 200062, China
[2]Texas AgriLife Research Mariculture Laboratory at Port Aransas, Texas A & M University, Port Aransas, TX 78373 USA

A B S T R A C T

A growth trial was conducted with *Litopenaeus vannamei* to evaluate effects of dietary vitamin and mineral supplementation (VMS) and water exchange on survival, growth and water quality. Four levels (0, 25, 50 and 100%) of VMS were evaluated using a 20% protein base diet. Postlarvae weighing 0.22 g were stocked for 26 days with either zero or high (5440% daily) water exchange. Growth was greater at zero than high exchange. However, growth was not affected by the level of VMS at both high and zero exchange. Survival for 0% VMS was lower than survivals for 25 to 100% VMS at high exchange. For 0% VMS, survival at high exchange was lower than survival at zero exchange. Results suggested that at zero water exchange, diets without VMS can replace diets with VMS without reducing survival.

Keywords: Litopenaues vannamei, vitamin, mineral, zero-water exchange, survival, growth

1. Introduction

Vitamin and mineral premixes are usually added to commercial shrimp diets (Akiyama et al., 1992). In addition to providing minimal levels for high growth and survival, these premixes are intended to replace vitamin and mineral losses associated with feed processing, feed storage and leaching in water. For vitamins, there is quantitative information on dietary requirements of individual vitamins. Using ascorbyl-2-polyphosphate, the requirement for vitamin C activity has been reported from 63 mg/kg (Castille et al., 1996) to 120 mg/kg of diet (He and Lawrence, 1993a). The requirement for vitamin E has been reported as 100 mg/kg of diet (He and Lawrence, 1993b). For minerals, dietary essentiality of copper (Cu) has been demonstrated by the observation of deficiency symptoms with diets containing less than 34 mg Cu/kg of diet (Davis et al., 1993a). For zinc (Zn), a requirement of 33 mg Zn/kg of diet was found to maintain normal tissue mineralization in the absence of phytate. However, in the presence of 1.5% phytate, 218 mg Zn/kg of diet was needed to satisfy the Zn requirement (Davis et al., 1993). For manganese (Mn), Davis et al. (1992) reported that dietary deletion reduced tissue mineralization in *Penaeus vannamei*, but had no effects on survival and growth. The vitamin and mineral supplements used in experimental research shrimp diets at the Texas AgriLife Research Mariculture Laboratory (Port Aransas, Texas, USA) are two Zeigler vitamin and mineral premixes (Zeigler Bros. Inc., Gardners PA, USA) and a stabilized form of vitamin C, ascorbyl-2-polyphosphate (Ju et al., 2012). The premixes contain 11 vitamins and 3 minerals, and 11 vitamins and one mineral, respectively (Table 1).

Aquaculture production of *L. vannamei* is currently limited by its environmental impact, the incidence of disease and the availability and quality of protein in dietary ingredients used in shrimp diets (Browdy et al., 2001; De Schryver et al., 2008; Hopkins et al., 1995). These challenges to production have led to development of zero water exchange shrimp culture technology. Generally present in zero water exchange systems are suspended particles which consist of a variety of microbes, microalgae, protozoa and other organisms together with detritus and dead organic matter (Avnimelech, 2012; Moeckel et al., 2012). These particles are collectively known as biofloc. Heterotrophic bacteria in biofloc can lower levels of ammonium and nitrite in culture systems (Asaduzzaman et al., 2008; Crockett et al., 2013). Biofloc can also indirectly control pathogenic bacteria by reducing infection and the spread of diseases through reduced water exchange (Cohen et al., 2005; Horowitz and Horowitz, 2001). Biofloc can improve production by providing a food source for shrimp and provide economic benefits by decreasing dietary requirements (Browdy et al., 2001; Hop-

⋆ Corresponding author email: smpall@yahoo.com

Table 1. Ingredient compositions of Zeigler vitamin-mineral premixes.

Ingredients	Units	Vitamin-mineral premix 1	Vitamin-mineral premix 2
Retinol; A	IU/kg	600000	1100000
Cholecalciferol; D	IU/kg	500000	500000
Tocopherol; E	mg/kg	40000	40000
Thiamine; B1	mg/kg	7000	3500
Riboflavin; B2	mg/kg	11000	5500
Pyridoxine; B6	mg/kg	22000	11000
Niacin	mg/kg	22000	11000
Pantothenic Acid	mg/kg	8000	4000
Biotin	mg/kg	200	100
Folic Acid	mg/kg	5000	2500
Cyanocobalaimine; B12	mg/kg	40	20
Zinc	mg/kg	46000	0
Manganese	mg/kg	1100	5300
copper	mg/kg	12000	0

kins et al., 1995). Some researchers have reported that biofloc can be consumed by shrimp and may lower the dietary protein levels required for production (Megahed, 2010; Wasielesky et al., 2006; Xu et al., 2012a) . However, information on the nutritional contribution of biofloc to dietary vitamin and mineral requirements is limited. Velasco and Lawrence (2000) reported that for *L. vannamei* in small tanks without water exchange, supplemental vitamins could be deleted.

Although the zero water exchange biofloc technology for shrimp production has been studied and developed, much is still unknown, particularly, management and maintenance of optimum biofloc levels and populations. With respect to shrimp growth and survival and water quality, little information exists on the interaction of effects of water exchange and shrimp dietary vitamin and mineral requirements. This study was conducted to investigate the effects of reducing dietary vitamin and mineral supplementation (VMS) at either zero or high water exchange in a growth trial stocked with *L. vannamei*. Effects of water exchange on reducing VMS were evaluated in terms of shrimp survival, growth and water quality.

2. Materials and methods

2.1. Experimental diets

Four semi-purified diets were prepared to contain 0, 25, 50 and 100% of the amount of VMS normally used in Texas AgriLife diets. VMS was reduced by replacement of vitamins and minerals with wheat starch. Ingredient compositions for the experimental diets are shown in Table 2.The calculated proximate composition and gross energy of all diets was 20% crude protein, 18.1% ash, 8.1% crude lipid, 3.3% fiber and $3,809$ cal/g. Calculated levels of Cu, Zn, Mn and individual vitamins in the experimental diets are shown in Table 3. Dry ingredients, including the binder, were mixed for a minimum of 40 minutes. Soybean and menhaden fish oils were gradually added and mixed for an additional 30 minutes. Water (40% of dry ingredients) was added to other mixed ingredients to form a dough, and then immediately extruded at room temperature through a 2 mm die using a Hobart A200 extruder (Hobart Corporation, Troy, New Jersey, USA). Extruded diets were dried at 25°C for 24h and then milled and sieved to obtain appropriate sizes for automatic feeders and the size of shrimp (Table 4). All diet was stored at -10°C in sealed plastic bags until the day of use.

Table 2. Ingredient compositions of the experimental diets

Ingredients	Vitamin and mineral supplementation (VMS) (% as fed basis)			
	0%		25%	50%
Wheat starch[a]	45.60	45.46	45.34	45.10
Vitamin-mineral premix 1 [b]	0.00	0.07	0.13	0.25
Vitamin-mineral premix 2 [b]	0.00	0.06	0.11	0.21
Stay C (ascorbyl-2-polyphosphate) 35%[b]	0.00	0.01	0.02	0.04
Squid muscle meal[b]	19.30	19.30	19.30	19.30
Fish meal, menhaden[c]	6.00	6.00	6.00	6.00
Methionine[h]	0.20	0.20	0.20	0.20
Menhaden fish oil[c]	1.40	1.40	1.40	1.40
Soybean oil[a]	0.70	0.70	0.70	0.70
Diatomaceous earth[a]	3.40	3.40	3.40	3.40
Calcium diphosphate[a]	6.70	6.70	6.70	6.70
Calcium carbonate[a]	0.90	0.90	0.90	0.90
Potassium chloride, reagent grade[g]	2.20	2.20	2.20	2.20
Sodium chloride, reagent grade[a]	1.60	1.60	1.60	1.60
Lecithin, dry,95%[f]	4.00	4.00	4.00	4.00
Cellulose[e]	3.20	3.20	3.20	3.20
Alginate [d]	3.00	3.00	3.00	3.00
Magnesium oxide[a]	1.60	1.60	1.60	1.60
Cholesterol[f]	0.20	0.20	0.20	0.20

2.2. Shrimp

Postlarvae *L. vannamei* were obtained from Shrimp Improvement System, Inc. (Islamorada, Florida, USA). Shrimp were fed a commercial diet (Zeigler Bros. Inc., Gardners, PA, USA) until stocked in the growth trial.

2.3. Experimental system

In the experiment, postlarval shrimp were stocked in tanks (bottom area 0.1 m^2, depth 0.2 m) for a 26-day growth trial. Water in each tank was aerated with a single $4\times2\times2$ cm air-stone to keep dissolved oxygen (DO) above 5 mg/l without water exchange, and to keep biofloc particles suspended. Aeration volume was 1 L min-1 at a depth of 0.2 m. Treatments in the experiment included two independent variables, VMS (0, 25, 50 and 100%) and water exchange (zero and high exchange). Water in high exchange tanks consisted of treated (mechanical, biological filtration and ultraviolet sterilization) water from a recirculating seawater system. Exchange of seawater in the culture tanks was 5440% per day. Each treatment contained six replicate tanks. Ten shrimp were randomly stocked into each tank, which was equivalent to 100 shrimp per m^2 or 500 shrimp per m^3. A photoperiod of 12-h light and 12-h dark was used.

2.4. Growth trial

For the growth trial, average weight at stocking (IBW) was 0.22 g $\pm$ 0.02 (SD) for $N = 48$. Differences between treatments were not significant ($P = 0.8489$). Automatic feeders fed shrimp 15 times daily to slight excess. At high exchange, uneaten diet and wastes were removed daily before filling feeders. Feeding rates and feed particle sizes are shown in Table 4.

2.5. Water quality monitoring

During the experimental period, water temperature, salinity, and DO were measured daily in different culture tanks at each water exchange rate with an YSI 85 oxygen/conductivity instrument (YSI, Yellow Springs, Ohio, USA). Total ammonia nitrogen (TAN), nitrite nitrogen ($NO_2 - N$), nitrate nitrogen ($NO_3 - N$), pH and alkalinity (KH) were measured once a week in three replicate tanks at each VMS for zero exchange and in one replicate tank at each VMS for high exchange. TAN, $NO_2 - N$ and $NO_3 - N$ were measured with a Hach DR/2100 spectrophotometer (Hach, Loveland, Colorado, USA) following the Standard methods for the examination of water and wastewater (APHA, 2005). pH was measured with a pH52 meter (Mil-

Table 3. Calculated levels of zinc, manganese, copper and vitamins in the experimental diets.

Vitamin or mineral (mg/kg)	Vitamin and mineral supplementation (VMS) (% as fed basis)			
	0%	25%	50%	100%
Retinol; A (IU kg^{-1})	0	387	773	1546
Cholecalciferol; D (IU kg^{-1})	0	324	649	1297
Tocopherol; E	0	55	109	218
Ascorbic acid; C	0	35	70	140
Thiamine; B1	0	7	13	26
Riboflavin; B2	0	10	20	40
Pyridoxine; B6	0	20	41	81
Niacin	0	21	42	83
Pantothenic Acid	0	8	15	30
Biotin	0	0.18	0.37	0.73
Folic Acid	0	5	9	18
Cyanocobalaimine; B12	0	0.04	0.08	0.15
Zinc	45	52	103	162
Manganese	25.7	16.3	32.6	39.5
Copper	10.9	11.7	23.4	35.9

waukee Instruments, Rocky Mount, North Carolina, USA). KH was measured by buret titration method (APHA, 2005).

2.6. Calculations and statistics

At the end of feeding trial, the number and final group weight of surviving shrimp were recorded for each culture tank. Performance parameters were final body weight (FBW), weight gain (WG) and survival. FBW = total weight/number of surviving shrimp, $WG = FBW - IBW$ and Survival$(\%) = 100 \times$ (number of surviving shrimp/number of stocked shrimp).

Temperature, salinity and DO were compared between high and zero exchange by one-way ANOVA. For each sample day, TAN, $NO_2 - N$, $NO_3 - N$, pH and KH were analyzed using one-way ANOVA of all VMS in high and zero exchange. Calculated growth and survival parameters were analyzed using two-way ANOVA. Student-Newman-Keuls(SNK) multiple range test was used to determine differences $(P < 0.05)$ among treatment levels. All statistical analyses were performed using the SAS microcomputer software package v9.3 (SAS Institute, Cray, North Carolina, USA).

3. Results

3.1. Shrimp performance

Growth (FBW and WG) and survival of *L. vannamei* fed the 0, 25, 50 and 100% VMS diets at high and zero exchange are given in Table 5 and Fig. 1. For growth parameters, interactions between diets and water exchange were not significant $(P \geqslant 0.3762)$. Growth was greater at zero than high exchange $(P \leqslant 0.0001)$. Differences in growth between diets were not significant $(P \geqslant 0.1593)$. In contrast to growth parameters, the interaction of survival between diets and water exchange was significant $(P < 0.0307)$. For zero exchange, one-way ANOVA indicated that survival (93-100%) did not differ between levels of VMS $(P = 0.5743)$. However, for high exchange, one-way ANOVA indicated that differences in survival were significant $(P = 0.0090)$. *A posteriori* comparisons of

Table 4. Feeding rates and feed particle sizes for the growth trial.

Day	Feed/shrimp (g)	Feed size[1]
1	0.084	20/18
2	0.103	18/14
3	0.122	18/14
4	0.140	18/14
5	0.159	18/14
6	0.178	14/12
7	0.187	14/12
8	0.187	14/12
9	0.193	14/12
10	0.193	14/12
11	0.211	14/12
12	0.211	14/12
13	0.211	14/12
14	0.232	14/12
15	0.232	14/12
16	0.232	14/12
17	0.232	14/12
18	0.255	14/12
19	0.255	12/7
20	0.255	12/7
21	0.280	12/7
22	0.280	12/7
23	0.280	12/7
24	0.308	12/7
25	0.308	12/7
26	0.353	12/7

[1] Feed between upper sieve number / below sieve number. U.S.A. Standard Testing Sieve. A.S.T.M.E-11 Specification. No.20: Opening micrometer 850m. No.18: Opening millimeter 1.00mm. No.14: Opening millimeter 1.40mm. No.12: Opening millimeter 1.70mm. No.7: Opening millimeter 2.80mm.

means for high exchange (Table 5) indicated that survival for 0% VMS (73.3%) was lower than survivals for 25 to 100% VMS (93 to 100%), and that survival did not differ between 25 and 100% VMS. For 0% VMS, survival at high exchange was lower than survival at zero exchange (Fig. 1).

3.2. Water quality

DO was lower ($P = 0.0483$) in zero exchange treatments (mean $\pm$ standard deviation of 5.75 ± 0.63 mg/L, $n = 24$) than in high exchange treatments (6.05 ± 0.34 mg/L, $n = 24$). Salinity was higher ($P < 0.0001$) in zero exchange treatments

Table 5. Effects of dietary vitamin and mineral supplementation (VMS) and water exchange on growth and survival for 26 day growth trial with *L. vannamei* stocked at 0.22 g $\pm$ 0.02 (SD). Values represent means $\pm SE$ for 6 replicates.

Water exchange	VMS (%)	FBW (g)[1]	WG (g)[1]	Survival (%)
High	0	1.79±0.10	1.58±0.10	78.3±7.49[B, 2]
	25	1.65±0.19	1.43±0.18	100±0.00[A]
	50	1.79±0.09	1.57±0.09	98.3±1.67[A]
	100	1.96±0.07	1.74±0.06	93.3±4.22[A]
Zero	0	2.58±0.10	2.37±0.10	98.5±1.52
	25	2.83±0.14	2.63±0.14	98.3±1.67
	50	2.76±0.04	2.55±0.04	100±0.00
	100	2.93±0.15	2.71±0.15	93.3±6.67
ANOVA, Pr >F				
VMS		0.1704	0.1593	0.0262
Exchange		<0.0001	<0.0001	0.0804
VMS × Exchange		0.4487	0.3762	0.0307

1 FBW: final body weight; WG: weight gain.
2 For survival at high water exchange, significant differences within treatments are indicated with different superscripts (Oneway ANOVA by $VMS, SNKP < 0.05$).

$(38.6 \pm 1.03$ ppt, $n = 24)$ than in high exchange treatments $(36.9 \pm 1.03$ ppt, $n = 24)$. Temperature was lower $(P = 0.0109)$ in zero exchange treatments $(27.4 \pm 1.9^oC, n = 24)$ than in high exchange treatments $(28.8 \pm 1.9^oC, n = 24)$.

Weekly means and standard errors of TAN, $NO_2 - N$ and $NO_3 - N$ are shown in Fig. 2. Water quality differences between diets were not significant at high and zero exchange. Values for diets at high exchange were pooled and shown as high exchange. Values for diets at zero exchange were pooled and shown as zero exchange. At zero exchange, TAN increased with time from day 12 through day 25 but did not exceed 0.19 mg/L. $NO_2 - N$ level increased with time to a maximum of 0.24 mg/L at day 25. $NO_3 - N$ level increased with time from day 17 through day 25 to a maximum of 61.9 mg/L at day 25. As expected, TAN, $NO_2 - N$ and $NO_3 - N$ levels were lower at high than zero exchange.

Weekly means and standard errors of pH and KH are shown in Fig.3 for pooled VMS diets at both zero and high exchange. Although pH decreased with time during the trial for zero exchange, it did not fall below 7.72. During the trial, KH remained between 154 and 200 mg/L for zero exchange.

4. Discussion

In this experiment, all shrimp were fed an excess amount of feed. This is verified by the high feed to weight gain ratios from 2.12 to 4.81. In addition, the quality of the shrimp and culture conditions used in the growth trial were adequate to detect treatment effects. For the 100% VMS diet at high exchange, in which culture conditions were adequate for high growth and survival, survival was 93.3% and the weight increase was 791% of the stocking weight.

In this study, growth was greater at zero exchange than at high exchange for all VMS levels. In addition, growth did not differ between VMS levels. Since there was no interaction between exchange and level of VMS, the greater growth at zero exchange was not caused by VMS. For this study, all diets contained 20%

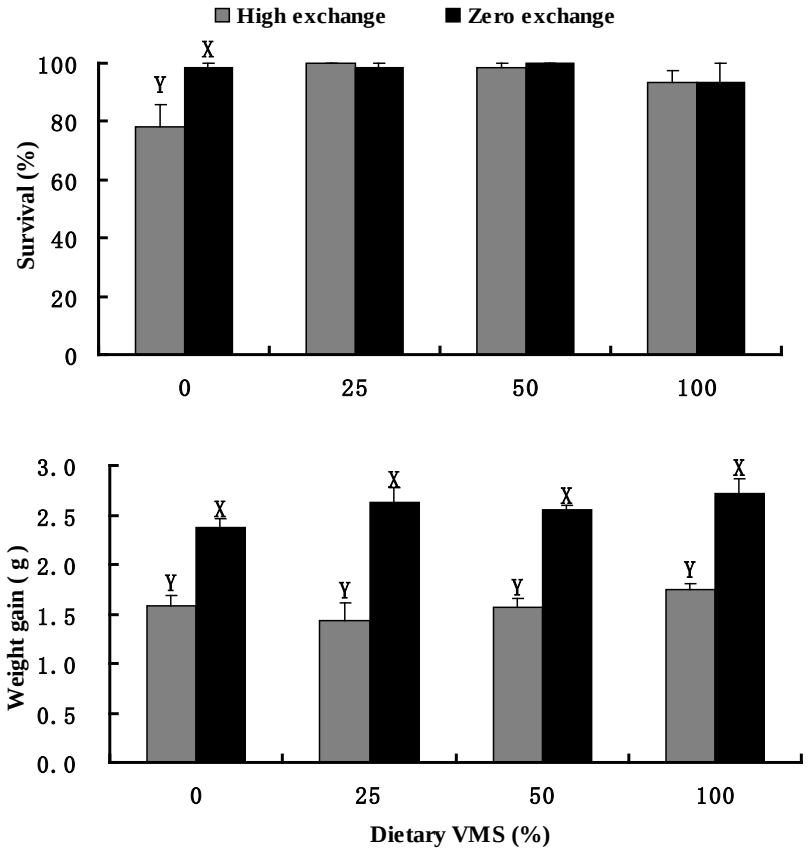

Figure 1. Effects of dietary vitamin and mineral supplementation (VMS) and water exchange on survival and weight gain (WG) for 26 day growth trial with *L. vannamei* stocked at $0.22g \pm 0.02$ (SD). Values represent means $\pm SE$ for 6 replicates. Significant differences between water exchange within each level of VMS are indicated with different letters (Oneway ANOVA, SNK $P < 0.05$).

protein because this level was adequate for maximum growth at zero water exchange. It is likely that the lower growth observed at high exchange was due to an inadequate dietary protein level for high exchange.

One explanation for enhanced growth at low water exchange is that biofloc developed in zero exchange culture tanks, and that shrimp were able to utilize the nutritional value of the biofloc. Improved growth and feed utilization in the presence of biofloc has been reported for *L. vannamei* (Wasielesky et al., 2006; Xu et al., 2012a; Xu and Pan, 2012b; Xu et al., 2013), *P. monodon* (Arnold et al., 2009), *P. semisulcatus* (Megahed, 2010) and *F. brasiliensis* (Emerenciano et al., 2012). Biofloc has been suggested to provide a supplemental food source to shrimp (Burford et al., 2004; Kuhn et al., 2008; Megahed, 2010). Biofloc can be consumed by cultured shrimp and provide important sources of nutrients (Burford et al., 2003; 2004; Tacon et al., 2002; Wasielesky et al., 2006; Xu et al., 2012a; Xu and Pan, 2012b; Xu et al., 2013). Moreover, biofloc, which exhibits high protease and amylase activities (Xu and Pan, 2012b), can contribute to digestion and utilization of shrimp diet. In addition, biofloc can stimulate production of digestive enzymes in shrimp (Xu et al., 2012a; Xu and Pan, 2012b; Xu et al., 2013).

In this study, high turbidity and brown color in zero exchange culture tanks suggested the presence of biofloc. Although culture tanks were not inoculated with biofloc prior to stocking, biofloc developed rapidly and visual observations of shrimp on the bottom of culture tanks were impossible within one week of stocking. Even though biofloc density was not quantified, and composition was not determined in this study, it is unlikely that biofloc density, composition and nutritional value were stable throughout either growth trial. Nonetheless, growth was clearly enhanced at zero exchange in this trial.

In contrast to growth, there was an interaction in this study between the effects of exchange and level of VMS on survival. At high exchange, survival with 0% VMS (78.3%) was lower than survival with 25 to 100% VMS (93.3 to 100%). Reduced survival without depression of growth for 0% VMS at high exchange was consistent with results reported by He and

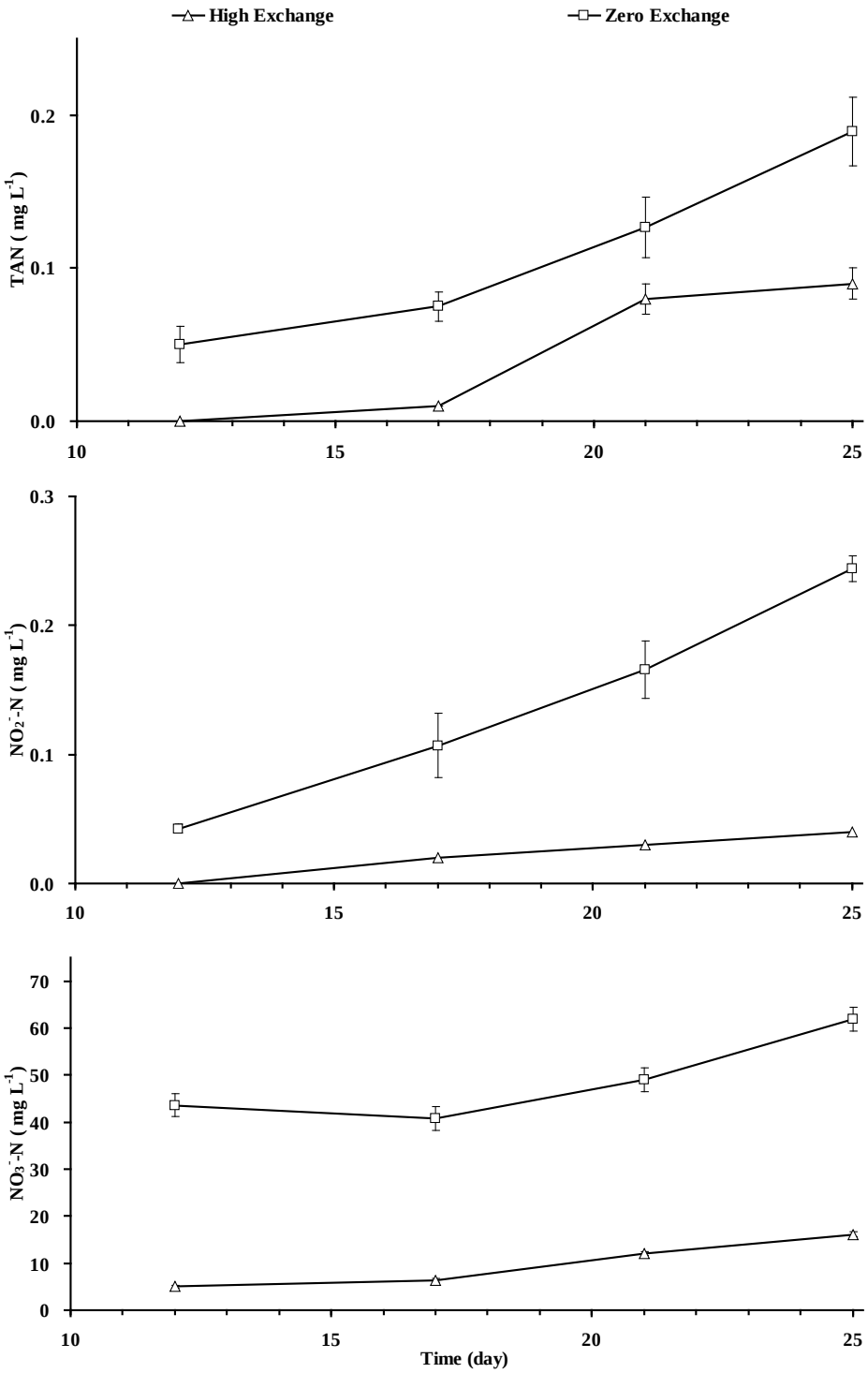

Figure 2. Effects of dietary vitamin and mineral supplementation (VMS) on levels of total ammonia nitrogen (TAN), nitrite nitrogen $(NO_2 - N)$ and nitrate nitrogen $(NO_3 - N)$ in 26 day growth trial with *L. vannamei* stocked at 0.22 g $\pm$ 0.02 (SD). For zero exchange, values are combined means $(\pm S.E)$ of three replicate tanks per sampling time of all VMS $(n = 9)$. The high exchange represents combined observations per sampling time of all VMS at high water exchange $(n = 3)$.

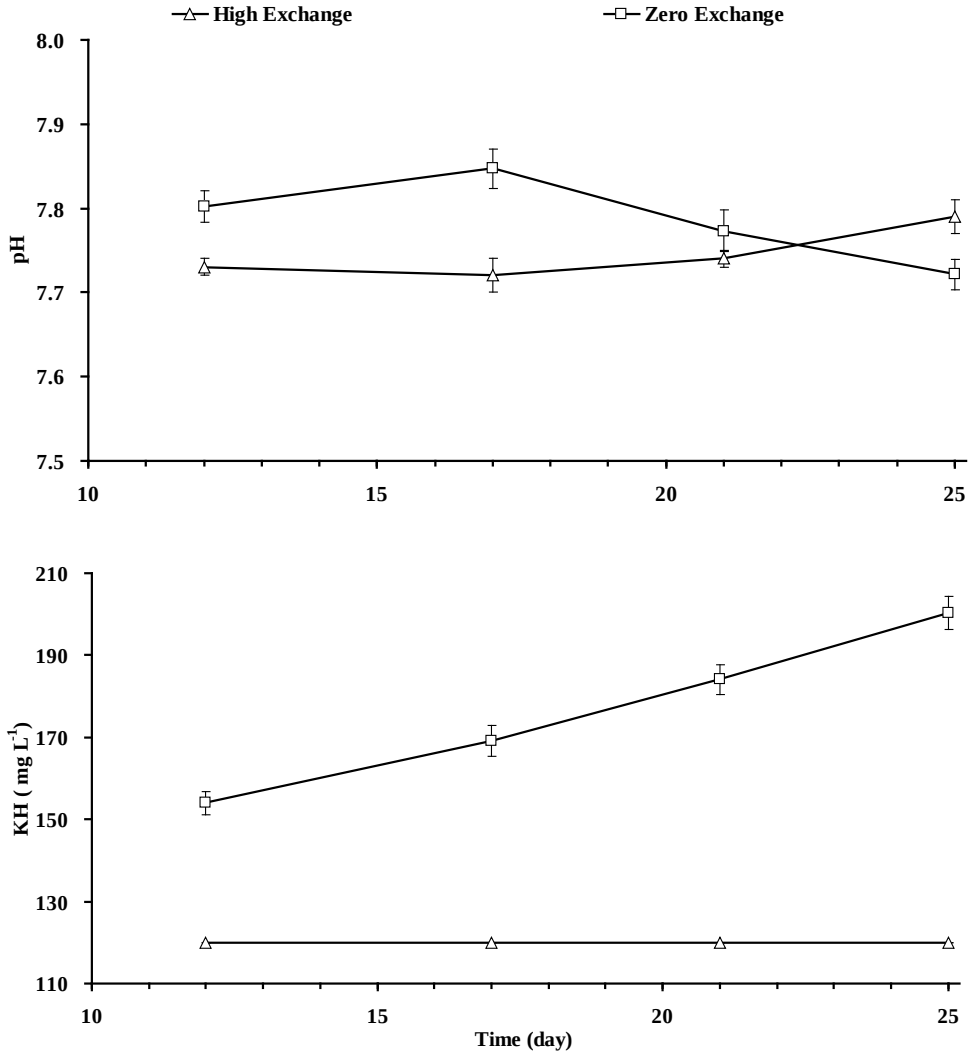

Figure 3. Effects of dietary vitamin and mineral supplementation (VMS) on pH and total alkalinity (KH) in 26 day growth trial with *L. vannamei* stocked at 0.22 g $\pm$ 0.02 (SD). For zero exchange, values are combined means $(\pm S.E)$ of three replicate tanks per sampling time of all VMS $(n = 9)$. The high exchange represents combined observations per sampling time of all VMS at high exchange $(n = 3)$.

Lawrence (1993a) and Castille et al. (1996) for *L. vannamei* diets without ascorbly-2-polyphosphate supplementation.

In contrast to high exchange, survival at zero exchange did not differ between levels of VMS. The absence of reduced survival with 0% VMS at zero exchange indicated that VMS may not be required at zero exchange. An explanation for this absence of an effect on survival is that biofloc in the zero exchange culture tanks may have provided necessary vitamins and minerals that were not available in the high exchange culture tanks. Tacon et al. (2002) reported that nutritional analysis revealed that biofloc was a good source of essential minerals and trace elements, and that supplemental vitamins in shrimp diets could be completely omitted in small outdoor tanks used for feeding trials. Velasco and Lawrence (2000) reported that L. vannamei survival and growth were not affected by diets with vitamin mixture levels from 0 to 0.5% in indoor tanks without water exchange.

In this study, salinity was higher, DO was lower and tem-

perature was lower in zero exchange tanks than in high exchange tanks. Higher salinity and lower DO in zero exchange have been respectively attributed to evaporation and higher respiration rates due to the presence of heterotrophic communities (Emerenciano et al., 2012). In this study, where enhanced growth was observed in treatments with zero exchange, the increased growth could not be attributed to differences in salinity, DO or temperature because all of these parameters were more conducive to growth at high exchange than at zero exchange.

In this study, water quality was potentially more limiting at zero than high water exchange. At zero exchange, levels of TAN, $NO_2 - N$ and $NO_3 - N$ were below 0.19, 0.24 and 61.9 mg/L, respectively. Levels of pH and KH were above 7.72 and 154 mg/L, respectively. All water quality parameters were adequate for optimal growth and survival.

5. Conclusions

In zero water exchange culture tanks, VMS was reduced in a low protein shrimp diet without reducing growth and survival. For the conditions of this growth trial, shrimp grown on a 20% protein diet without VMS with zero water exchange had higher growth and higher survival than shrimp fed a 20% protein diet with VMS with high water exchange. For 0% VMS, survival at high exchange was lower than survival at zero exchange. Results suggested that at zero water exchange, diets without VMS can replace diets with VMS without reducing survival.

6. Acknowledgements

The research was funded by Project R-9500, Texas A&M AgriLife Research, Texas A&M University System and China Scholarship Council. The authors also would like to acknowledge Jack Crockett, Jessica Morgan and Ivy McClellan for reviewing this publication.

References

Akiyama, D.M., Dominy, W.G., and Lawrence, A.L. 1992.Penaeid shrimp nutrition, *in* Fast, A.W., Lester, L.J. (eds.), *Marine Shrimp Culture: Principles and Practices*. Elsevier, Amsterdam, 535568.

APHA, 2005. *Standard Methods for the Examination of the Water and Wastewater,* 21st ed.) American Public Health Association, Washington, DC.

Arnold, S.J., Coman, F.E., Jackson, C.J., and Groves, S.A. 2009. High-intensity, zero water exchange production of juvenile tiger shrimp, *Penaeus monodon*: an evaluation of artificial substrates and stocking density. *Aquaculture* **293**, 42–48.

Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Huque, S., Salam, M.A., and Azim, M.E. 2008. C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture+* **280**, 117–123.

Avnimelech, Y. 2012. *Biofloc Technology A Practical Guide Book*, 2d Edition. The World Aquaculture Society, Baton Rouge.

Browdy, C.L., Bratvold, D., Stokes, A.D., and Mcintosh, R.P. 2001. Perspectives on the application of closed shrimp culture systems, *in* E.D. Jory & C.L. Browdy (eds), *The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture*, The World Aquaculture Society, Baton Rouge, 20–34.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., and Pearson, D.C. 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture.* **219**, 393–411.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., and Pearson, D.C. 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture* **232**, 525–537.

Castille, F.L., Lawrence, A.L., Seib, P.A., and Wang, X.Y. 1996. Effect of ascorbyl-2-polyphosphate on survival, growth and tissue ascorbic acid in the shrimp, *Penaeus vannamei* Boone 1931. The World Aquaculture Society, Bangkok, 65.

Cohen, J., Samocha, T.M., Fox, J.M., Gandy, R.L., and Lawrence, A.L. 2005. Characterization of water quality factors during intensive raceway production of juvenile *L. vannamei* using limited discharge and biosecure management tools. *Aquacultural Engineering* **32**, 425-442.

Crockett, J., Lawrence, A.L., and Kuhn, D.D. 2013. Shallow nursery system uses bioreactor concept for production of juvenile shrimp. *Global Aquaculture Advocate* **16(3)**, 72–75.

Davis, D.A., Lawrence, A.L., and Gatlin, D.M. 1992. Mineral requirements of *Penaeus vannamei*: A preliminary examination of the dietary essentiality for thirteen minerals. *Journal of the World Aquaculture Society* **23(1)**, 8–14.

Davis, D.A., Lawrence, A.L., and Gatlin, D.M. 1993a. Dietary copper requirement of *Penaeus vannamei*. *Bulletin of the Japanese Society of Science and Fisheries* **59**, 117–122.

Davis, D.A., Lawrence, A.L., and Gatlin, D.M. 1993b. Dietary zinc requirement of *Penaeus vannamei* and the effects on phytic acid and phosphorus bioavailability. *Journal of the World Aquaculture Society* **24**, 40–47.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., and Verstraete, W. 2008. The basics of bioflocs technology: the added value for aquaculture. *Aquaculture* **277**, 125–137.

Emerenciano, M., Ballester, E.L.C., Cavalli, R.O., and Wasielesky, W. 2012. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture Research* **43**, 447–457.

He, H. and Lawrence, A.L. 1993a. Vitamin C requirements of the shrimp *Penaeus vannamei*. *Aquaculture* **114**, 305–316.

He, H. and Lawrence, A.L. 1993b. Vitamin E requirement of *Penaeus vannamei*. *Aquaculture* **118**, 245–255.

Hopkins, J.S., Sandifer, P.A., and Browdy, C.L. 1995. Effect of two protein levels and feed rate combinations on water quality and production of intensive shrimp ponds operated without water exchange. *Journal of the World Aquaculture Society* **26**, 93–97.

Horowitz, A. and Horowitz, S. 2001. Disease control in shrimp aquaculture from a microbial ecology perspective., *in* C.L. Browdy and C.L. Jory (eds.), *Proceedings of the Special Session on Sustainable Shrimp Farming*, The World Aquaculture Society, Baton Rouge, 199-218.

Ju, Z. Y., Castille, F., Deng, D.F., Dominy, W.G., Lawrence, A.L., and Forster, I.P. 2012. Effects of replacing fish oil with stearine as main lipid source in diet on growth and survival of Pacific White Shrimp, *Litopenaeus vannamei*(Boone, 1931). *Aquaculture Research*

43, 1528–1535.

Kuhn, D.D., Boardman, G.D., Craig, S.R., Flick, G.J., and McLean, E. 2008. Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp, *Litopenaeus vannamei*, in recirculating aquaculture systems. *Journal of the World Aquaculture Society* **39**, 72–82.

Megahed, M.E. 2010 The effect of microbial biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus Semisulcatus*) fed with different crude protein levels.. *Journal of the Arabian Aquaculture Society* **5**, 119–142.

Moeckel, J.L., Lawrence, A.L., Crockett, J., Lingenfelter, B.A., Patnaik, S., and Pollack, J.B. 2012. Effect of Dietary Protein on Growth and Survival of Juvenile *Litopenaeus vannamei* in a Zero Water (Biofloc) Exchange System. *9th International Conference on Recirculating Aquaculture* 173-210.

Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., and Decamp, O.E. 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition* **8** 121–137.

Velasco, M., and Lawrence, A.L. 2000. Initial evaluation of shrimp vitamin requirements in laboratory tanks without water exchange. *Global Aquaculture Advocate* **3(4)**, 23.

Wasielesky, W., Atwood, H., Stokes, A., and Browdy, C.L. 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture* **258**, 396-403.

Xu, W.J., Pan, L.Q., Zhao, D.H., and Huang, J. 2012a. Preliminary investigation into the contribution of bioflocs on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks. *Aquaculture* **350-353**, 147–153.

Xu, W.J., and Pan, L.Q. 2012b. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture* **356-357**, 147–152.

Xu, W.J., Pan, L.Q., Sun, X.H., and Huang, J. 2013. Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. *Aquaculture Research* **44(7)**, 1093-1102.