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Effects of dietary phosphorus level and inorganic source on survival and growth of *Penaeus vannamei* postlarvae in zero-water exchange culture tanks

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Abstract – Zero-water exchange culture tanks were stocked with *Penaeus vannamei* postlarvae to compare the effects of (i) three dietary phosphorus levels: 0.4, 0.8 and 1.2 %, and (ii) three dietary inorganic phosphorus sources: $CaHPO_4$, Na_2HPO_4 and NaH_2PO_4, on postlarvae biological performance, and total reactive phosphorus accumulation in the water (TRPAW). Dietary Ca:P ratio was maintained within a 1:1 to 1:2 ratio. Postlarvae survival was high and not significantly different among treatments. Postlarvae growth was not significantly different, regardless of dietary phosphorus level. TRPAW was significantly higher with increasing level of dietary phosphorus. Dietary inorganic phosphorus source did not have a significant effect on postlarvae growth. No significant differences were found on TRPAW between diets with Na_2HPO_4 and NaH_2PO_4 supplementation at equal dietary phosphorus level. TRPAW was significantly lower with $CaHPO_4$ than with Na_2HPO_4 or NaH_2PO_4 supplementation at equal dietary phosphorus level. Environmental quality of culture water may be greatly modified through nutritional strategies without negatively affecting shrimp biological performance. © Ifremer-Elsevier, Paris

postlarvae / phosphorus / water quality bioassay / nutritional requirements / shrimp culture / *Penaeus vannamei*

Résumé – Effets du niveau de phosphore dans l'alimentation et du type de source minérale en phosphore sur la survie et la croissance des postlarves de *Penaeus vannamei* élevées en bassins et en milieu non renouvelé. Des postlarves de *Penaeus vannamei* sont stockées en bacs d'élevage en milieu non renouvelé afin de comparer les effets d'une supplémentation en phosphore, (i) dans les aliments à 0,4 ; 0,8 et 1,2 % et (ii) à partir de 3 sources minérales, $CaHPO_4$, Na_2HPO_4 et NaH_2PO_4 , sur les performances biologiques des postlarves et sur l'accumulation du phosphore total dans l'eau (TRPAW). Le rapport Ca:P est maintenu entre 1:1 et 1:2. La survie des postlarves est importante, sans différence significative entre les traitements. La croissance des postlarves n'est pas significativement différente en ce qui concerne le niveau de phosphore considéré dans l'alimentation. Le TRPAW augmente significativement avec le niveau de phosphore dans l'alimentation. Aucune différence significative n'a été trouvée, à niveau de phosphore équivalent dans l'alimentation sur TRPAW entre les régimes « supplémentés » en Na_2HPO_4 et NaH_2PO_4 ; en revanche, le phosphore total (TRPAW) est inférieur si la source en phosphore est $CaHPO_4$. La qualité du milieu d'élevage peut être modifiée de façon importante selon les stratégies nutritionnelles, sans pour autant affecter la survie et la croissance des crevettes. © Ifremer-Elsevier, Paris

postlarves / phosphore / qualité de l'eau / besoins nutritionnels / élevage de crevettes / *Penaeus vannamei*

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1. INTRODUCTION

Shrimp are able to assimilate minerals directly from the aquatic environment [13, 19]. However, phosphorus concentrations are usually very low [1] and consequently most of this mineral must be supplied in the feed. In shrimp, phosphorus is mainly found associated with calcium in the exoskeleton.

In addition, it is a component of phospholipids, high-energy compounds such as adenosine triphosphate (ATP), nucleic acids such as DNA and RNA, coenzymes, and metabolic intermediates. Phosphorus also plays a role as a pH regulator of intra- and extracellular fluids.

Early research with *Penaeus japonicus* indicated a dietary phosphorus requirement of 2 % with a Ca:P ratio of 1:1 [12]. Civera and Guillaume [7] found that a diet containing 0.6 % phosphorus was adequate for good survival and growth of *Penaeus japonicus* and *Penaeus vannamei.* Davis et al. [11] reported that in the absence of calcium supplementation, a 0.4 % phosphorus level in the diet supported good survival and growth of juvenile *Penaeus vannamei.* These authors also recommended a dietary Ca:P ratio of 1:1. It has been reported that postlarval shrimp have higher nutritional requirements than larger shrimp [5, 8]. Thus, it is possible that the dietary phosphorus requirement may be higher for postlarvae (PL) PL5-PL8 than the level found by Davis et al. [11] for PL27 *Penaeus vannamei.* Phosphorus in effluent discharge waters from aquaculture operations has been targeted as one of the polluting agents to natural habitats [2, 16]. Feeds have been identified as the major source of this element in the effluent water [9]. Thus, efforts should be directed not only to optimize the dietary phosphorus level and source for good survival and growth of shrimp but also to minimize loading in the culture water. The objectives of this study were (a) to determine the effect of three dietary phosphorus levels and three dietary inorganic phosphorus sources on survival and growth of *Penaeus vannamei* postlarvae, and (b) to measure total reactive phosphorus accumulation in the water of a zero-water exchange culture system.

2. MATERIALS AND METHODS

Composition of experimental semi-purified diets and mineral mixes is presented in *tables I* and *II.* Mineral mixes contained calcium phosphate dibasic ($CaHPO_4$), sodium phosphate dibasic (Na_2HPO_4), and sodium phosphate monobasic (NaH_2PO_4) as the inorganic source of phosphorus. Dietary phosphorus levels of 0.8 % with $CaHPO_4$, Na_2HPO_4, and NaH_2PO_4, and of 0.4, 0.8 and 1.2 % with NaH_2PO_4 were achieved (*table I*). A dietary Ca:P ratio between 1:1 and 1:2 was maintained in all diets. Krill meal was used as a feed attractant based on previous observations in our laboratory. Each diet was formulated to contain nominal values of 4.2 kcal intake energy·g^{-1}, 32 % crude protein, 6 % ether extract, 10 % ash and 3 % crude fibre on an as-fed basis. Experimental diets were prepared by mixing dry ingredients for 25 min in a twin shell dry V- blender (maximum capacity 4 kg, Patterson-Kelley Co., East Stroudsburg, Pennsylvania). The mixed ingredients were then transferred to a food blender (Model C-100, Hobart Manufacturing Corp., Troy, Ohio) and menhaden oil added into the mixture and mixed for 10 min. Lastly, 400 ml boiling deionized water was added per kg of diet and mixed for 5 min to achieve a proper consistency of the mash for pelleting with a food mixer (Model A-200, Hobart Manufacturing Corp., Troy, Ohio) through a 3-mm die. Pellets were oven-dried at 60 °C to a moisture content of 7-8 %, ground and sieved to 1.0-0.5 mm particle size, and stored at –10 °C until used. Feeds Ca, P, and proximate analysis were performed by a commercial labo-

Table I. Proximate composition and analysis of test diets (% as-fed basis).

Wheat starch[1]	43.6	43.6	43.6	43.6	43.6
Soybean protein isolate[1]	10.1	10.1	10.1	10.1	10.1
Wheat gluten[1]	7.1	7.1	7.1	7.1	7.1
Casein[2]	15.6	15.6	15.6	15.6	15.6
Menhaden fish oil[3]	4.0	4.0	4.0	4.0	4.0
Krill meal[4]	4.0	4.0	4.0	4.0	4.0
Lecithin[5]	1.0	1.0	1.0	1.0	1.0
Cholesterol[1]	0.5	0.5	0.5	0.5	0.5
Carboxymethylcellulose[2]	3.0	3.0	3.0	3.0	3.0
Diatomaceous earth[1,6]	6.3	1.3	1.3	1.3	1.3
Mineral mixture AIN 76[1,7]	4.0	0.0	0.0	0.0	0.0
Mineral mixture A[7]	0.0	9.0	0.0	0.0	0.0
Mineral mixture B[7]	0.0	0.0	0.0	9.0	0.0
Mineral mixutre C[7]	0.0	0.0	9.0	0.0	0.0
Mineral mixture D[7]	0.0	0.0	0.0	0.0	9.0
Stay-C (25 % active)[8]	0.3	0.3	0.3	0.3	0.3
Vitamin mixture[9]	0.5	0.5	0.5	0.5	0.5
Proximate analysis					
Crude protein	31.4	31.6	33.0	32.0	32.7
Crude lipid	5.7	5.7	5.7	5.7	5.6
Moisture	8.0	7.4	7.3	7.3	7.3
Ash	11.0	10.0	9.1	10.8	8.4
Calcium	0.7	0.7	0.7	0.2	1.1
Total phosphorus	0.8	0.8	0.8	0.4	1.2

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[2] United States Biochemicals, Cleveland, Ohio, USA.
[3] Zapata Haynei Corp., Reedville, Virginia, USA.
[4] Inual, Santiago, Chile.
[5] Central Soya, Fort Wayne, Indiana, USA.
[6] Acid washed.
[7] Table 2.
[8] L-Ascorbyl-2-Polyphosphate. Hoffman-LaRoche Inc., Nutley, New Jersey.
[9] Dawes Laboratories, Arlington Heights, Illinois, USA. Composition (g/kg): Retinol (22.2), Cholecalciferol (1.1), Tocopherol (10.3), Menadione (2.3), Thiamine (5.0), Riboflavin (5.7), Pyridoxine (10.1), Niacin (10.9), Pantothenic acid (10.9), Biotin (0.2), Choline (0.8), Folic acid (3.5), Cyanocobalamine (0.02), Dextrin (917.0).

Table II. Mineral sources and inclusion levels ($g \cdot kg^{-1}$) for mineral mixes.

	Mineral mixture				
	AIN 76[1]	A	B	C	D
Calcium phosphate dibasic	500				
Sodium phosphate dibasic		225			
Sodium phosphate monobasic			29	225	338
Calcium chloride		235	31	240	360
Sodium chloride	74		164	81	33
Diatomaceous earth		351	587	265	80
Other mineral components	426	189 [2]	189 [2]	189 [2]	189 [2]

[1] Mineral mixture AIN 76 composition ($g \cdot kg^{-1}$): calcium phosphate dibasic (500.0), chromium potassium sulfate (0.55), cupric carbonate (0.3), ferric citrate (6.0), magnesium oxide (24.0), manganous sulfate (3.5), potassium citrate monohydrate (220.0), potassium iodate (0.01), potassium sulfate (52.0), sodium chloride (74.0), sodium selenite (0.01), sucrose (118.0), zinc carbonate (1.6).
[2] Amounts of other mineral components on AIN 76 mineral mixture were reduced by 55.6 % on these mineral mixtures.

ratory (Woodson-Tenent Laboratories Inc., Memphis, Tennessee). Hatchery reared *Penaeus vannamei* postlarvae (PL) of 0.92 ± 0.04 mg average initial weight were stocked at 1.5 $PL \cdot L^{-1}$ in 20 L of water (stocking density related to surface area was 444 $PL \cdot m^{-2}$) in fibreglass tanks. Postlarvae were fed every 96 min with automatic feeders during the 2 one-day experimental period according to the following feed curve [24], where y = feed/PL (in mg) and x = day on growth trial: days 1-16, $y = 1.555 - 0.116x + 0.086x^2 - 0.003x^3$, days 17-20 $y = -6.427 + 0.805x$. This feeding regime had proven effective in our laboratory under these culture conditions. Culture tanks were filled with seawater at the start of the experiment and no new water was added during the length of the growth trial. This water management practice was used as a more holistic approach compared to methods of high water renewal to investigate feed nutrient level and feed ingredient effects on shrimp biological performance and water quality in the laboratory. Seawater salinity was adjusted to 24 with fresh water. Water temperature was maintained at 27-29 °C. Aeration via a single airstone maintained water dissolved oxygen concentration above 4.0 $mg \cdot L^{-1}$ in each tank through out the experimental period. A photoperiod of 12 h light and 12 h dark was maintained by an automatic timer. Seawater temperature and dissolved oxygen were measured daily with a YSI meter (Model 58, Yellow Springs Inc., Yellow Springs, Ohio). Water salinity was measured every two days with a temperature-compensated refractometer (Model ABMTC, Aquafauna, Hawthored, California). Prior to shrimp harvest, water samples were taken from all tanks near the surface and close to the airstone where good water mixing ocurred. Samples were immediately analysed for total reactive phosphorus [18, 23], total ammonia-N [22, 23], nitrite-N [21, 23] and nitrate-N [17, 23]. Total reactive phosphorus was determined by directly analysing the water sample without filtration or digestion [14]. An spectrophotometer (Model spectronic 401, Milton-Roy Co., Rochester, New York) was used to read water samples. A pH meter (Model 701A\digital ionalyzer, Orion Research Inc., Cambridge, Massachusetts) was used to measure pH. PL were air blotted, weighed and counted at the beginning and end of the experiment. PL survival, relative growth rate ([wet weight increase/initial weight] × 100 and feed conversion ratio) were calculated. Data obtained from this completely randomized design with 7 replicates per treatment were analysed using one-way analysis of variance to determine significant differences ($P < 0.05$) among treatment means. Tukey-Kramer Honest Significant Difference test for mean separation was used to evaluate significant differences among treatments [15]. All statistical analyses were performed using the Statistical Analysis System procedures (SAS Institute, Inc., Cary, North Carolina, 1989-91.JMP version 2.0.5).

3. RESULTS

Postlarvae survival was high and not significantly different regardless of dietary phosphorus level (*table III*) or inorganic phosphorus source (*table IV*). At the end of the growth trial average treatment concentrations of total ammonia-N and nitrite-N ranged from 0.21 to 0.28 (pH ranged from 7.7 to 8.0) and from 1.90 to 2.25 $mg \cdot L^{-1}$, respectively. No significant differences were found on dissolved total inorganic nitrogen (TIN = total ammonia-N + nitrite-N + nitrate-N) in the water. Average treatment concentrations of TIN ranged from 3.55 to 3.95 $mg \cdot L^{-1}$. Seawater salinity increased from 24 to 26 during the length of the growth trial. Growth of postlarvae fed on diets of different dietary phosphorus levels with NaH_2PO_4 supplementation was not significantly different (*table III*). However, total reactive phosphorus accumulation in the water

Table III. Biological performance of *Penaeus vannamei* postlarvae fed diets for 20 days with $N_aH_2PO_4$ supplementation and total reactive phosphorus accumulation in water (TRPAW). Entries are sample mean ± SD; 7 replicates per treatment. Values with different letters indicate significant differences.

Dietary phosphorus level (%)	0.4	0.8	1.2
Survival (%)	81.4 ± 8.8	84.3 ± 13.8	85.2 ± 8.8
Initial weight (mg)	0.92 ± 0.04	0.92 ± 0.04	0.92 ± 0.04
Final weight (mg)	126.48 ± 21.21	148.99 ± 29.98	143.28 ± 25.99
Relative growth rate (%)	13 573 ± 2 293	16 007 ± 3 134	15 390 ± 2 810
Feed conversion ratio	1.1	0.9	0.9
TRPAW ($mg \cdot L^{-1}$)	0.19 ± 0.05 [a]	0.94 ± 0.11 [b]	1.67 ± 0.26 [c]

(TRPAW) significantly increased with increased phosphorus levels (*table III*). Postlarvae growth was not significantly different, regardless of inorganic phosphorus source in the diet at a 0.8 % dietary phosphorus level (*table IV*). TRPAW was significantly lower with diets supplemented with $CaHPO_4$ than with Na_2HPO_4 or NaH_2PO_4. No significant differences were found on TRPAW between diets containing dibasic or monobasic forms of sodium phosphate (*table IV*).

Table IV. Biological performance of *Penaeus vannamei* postlarvae fed diets supplemented with three different inorganic phosphorus sources for 20 days at a 0.8 % dietary phosphorus level, and total reactive phosphorus accumulation in water (TRPAW). Entries are sample mean ± SD; 7 replicates per treatment. Values with different letters indicate significant differences.

Inorganic phosphorus source	$CaHPO_4$	Na_2HPO_4	NaH_2PO_4
Survival (%)	84.3 ± 8.9	87.6 ± 6.6	84.3 ± 13.8
Initial weight (mg)	0.92 ± 0.04	0.92 ± 0.04	0.92 ± 0.04
Final weight (mg)	123.79 ± 32.77	144.66 ± 20.64	148.99 ± 29.98
Relative growth rate (%)	13 283 ± 3 543	15 539 ± 2 231	16 007 ± 3 134
Feed conversion ratio	1.1	0.9	0.9
TRPAW ($mg \cdot L^{-1}$)	0.54 ± 0.20 [a]	1.05 ± 0.14 [b]	0.94 ± 0.11 [b]

4. DISCUSSION

Excellent postlarvae survival was obtained regardless of diet. Water quality values for total ammonia-N and nitrite-N in these trials were below those reported as toxic for *Penaeus monodon* [3, 4, 6]. Similar postlarvae growth achieved with the three levels of dietary phosphorus tested indicate that the lowest dietary phosphorus level of 0.4 % (Ca:P of 1:2) used in this experiment was adequate for good survival and growth of postlarval *Penaeus vannamei*. Survival and growth rates of postlarvae in this experiment were higher in most cases than those reported by Ogle [20] for postlarvae *Penaeus vannamei* under several indoor experimental conditions with initially larger animals and longer trial periods. Davis et al. [11] had reported that in the absence of calcium supplementation, a 0.36 % dietary phosphorus level was adequate for good growth of *Penaeus vannamei* juveniles. Our results indicated that even with some Ca present in the diet the required dietary phosphorus level is very low for postlarvae. The significant increase in total reactive phosphorus accumulation in the water (TRPAW) with increasing level of dietary phosphorus showed the impact that dietary phosphorus levels may have on water quality. Besides the potential economic benefit associated with a reduction in the amount of phosphorus in the feed, these data indicated that over supplementation of phosphorus in the diet may be avoided to decrease the level of TRPAW in the system culture water. Survival and growth were similar for postlarvae fed diets with different inorganic phosphorus sources at a dietary phosphorus level of 0.8 %. However, TRPAW was significantly lower with $CaHPO_4$ than Na_2HPO_4 or NaH_2PO_4. TRPAW was similar for the dibasic and monobasic forms of sodium phosphate. These data showed that the source of inorganic phosphorus in the diet also affected the TRPAW. Davis and Arnold [10] reported a lower phosphorus availability for shrimp from dibasic phosphorus sources compared to monobasic sources. The present study indicated that dibasic inorganic phosphorus forms may be more preferable to use in shrimp feeds when shrimp biological performance and TRPAW are considered concomitantly. Thus, dietary phosphorus level and source of inorganic phosphorus in shrimp feed formulations should be selected in terms of shrimp biological performance, cost, availability, and environmental impact in the culture water.

5. CONCLUSION

The data from this research indicated that postlarvae biological performance was satisfactory under these culture conditions regardless of dietary phosphorus level or dietary inorganic phosphorus source. However, feed composition greatly influenced total reactive phosphorus accumulation in the water. Use of zero-water exchange culture systems to evaluate different feed ingredients and nutrient levels may increase our knowledge of ingredient and nutrient levels that would provide maximum shrimp growth and minimum nutrient loading in the culture water.

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