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Total Nitrogen and Phosphorus loading in to aquatic environment from shrimp Penaeus monodon (L.) fed with commercial diets

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Abstract:

A study was undertaken to assess the artificial feed related waste accumulation in culture of shrimp Penaeus monodon. For this study, widely used five commercial shrimp diets (CD1 to CD5) were fed to shrimps, which were reared in 200 Lcapacity FRP tanks at the stocking density of 15 nos/tank for a period of 90 days. The diets were offered to shrimps at ad libatum @ four times per day. Food consumption of P. monodon varied from 6.96 ± 0.25 to 8.81 ± 0.31 g. FCR of P. monodon fed on CD5 diet was less and it was more for CD1 diet fed group. The SGR of all the diets fed groups ranged from 5.75 ± 0.25 to 6.25 ± 0.20% with the maximum at CD2 and CD5 diets fed groups. Maximum retention and minimum loss of nitrogen was registered in CD4 diet fed group. Phosphorus retention was low in CD1 diet fed group, but total phosphorus loss was low in CD4 diet fed group (33.05 mg). Total nitrogen and phosphorus loading were deviated much between test diets. The total nitrogen loading rate ranged between 23.48 to 45.54 kg t^{-1} shrimp production and the

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phosphorus loading rate ranged between 6.25 to 9.85 kg t^{-1} shrimp production.

Key words: Nitrogen; Phosphorus; *P.monodon*; commercial shrimp diets.

INTRODUCTION

Aquaculture, especially shrimp farming, has been the target of constant pressure from non-governmental organizations and environmental agencies, which report that the activity imposes considerable harm to coastal environments. However, adequate management strategies with regard to feeding, water quality and soil quality have contributed to make the activity sustainable (Maia et al., 2011; Brito and Olivera, 2010). Viral disease has taken a heavy toll in many countries, the production level of world farmed shrimp has increased to reach more than 1.5 million tonnes. Out of 40 million tonnes of world inland aquaculture production, shrimp alone contributing 3.5% and it has been predicted that in the year 2020, the global production will rely less on natural stocks and more on aquaculture (FAO 2004). In shrimp farming, commercial feeds are playing decisive role in enhancing larval survival and production target. Now-a-days, in shrimp culture, a variety of commercial feeds are used with varying levels of nutrients and also with essential additives including phosphorus. Similar to that of all other aquaculture practices, shrimp culture industry is also facing the problem of accumulation of feed related solid and soluble wastes. This poses the problem of accumulation of nitrogen and phosphorus in the culture system and also to the surrounding ecosystem (Weismann *et al.* 1988). It was estimated that 53 to 71kg and 20 to 23 kg of total nitrogen and phosphorus respectively were released into the water per tonne of carp production (Watanabe 1991). Environmental

phosphorus loading from carp fed commercial diets was reported to be around 20 kg / tonne of fish production on average in the case of cage culture at Lake Kasumigaura of Japan until the prefectural government established a new regulation (Watanabe *et al.* 1987a and 1999). In salmonids, the nitrogen accumulation was 3 kg/ tonne of fish production. The release of phosphorus and nitrogen is mainly influenced by the availability of dietary phosphorus and nitrogen (Rodehutscord 1996). Phosphorus and nitrogen are the key nutrients in causing excessive algal blooms and the eutrophication of natural waters (Environmental Protection Agency 1973). Though shrimp culture was undertaken extensively in Asian countries, data related to the accumulation of nitrogen and phosphorus is still wanted. Viewing this, the present study was undertaken on the nitrogen and phosphorus release in shrimp culture system used commercial diets.

MATERIALS AND METHODS

Collection of shrimps

For the present study, the post larvae (PL20) of tiger shrimp, *Penaeus monodon* were obtained from a commercial shrimp hatchery, Kulasekharapatinam, Tuticorin District, Tamil nadu. The post larvae were transported in oxygenated bags with least disturbance and acclimatized to the ambient laboratory condition (salinity 20 ppt; temperature 28 ± 1^0C; pH 8.0 ± 0.2) in one tonne capacity FRP tank for a period of 5 days and were then used for rearing experiment.

Experimental diets

Five commercial shrimp diets (CD1, CD2, CD3, CD4 and CD5) were selected for the present investigation. In all these commercial diets, grower, starter and finisher feeds were used. The biochemical constituents such as protein (Lowry *et al.*

1951), carbohydrate (Seifter *et al.* 1950), lipid (Folch *et al.* 1957) and total phosphorus (APHA 1985) contents of all the types of experimental diets were measured. For calculating nitrogen and phosphorus budget, the biochemical constituents of starter, grower, finisher feeds of the individual commercial diet was pooled together and the average value of the respective constituents was then taken. The energy density of the experimental diets was also estimated using PARR (Moline,USA) 1421 semi micro bomb calorimeter. Considering the protein and energy value of the particular diet, P: E ratio was calculated.

Feeding experiment

Healthy shrimps (PL 25) having an average body weight of 21.80 ± 0.98 mg were randomly distributed in 200 l capacity FRP tanks containing 150 l water having 20 ppt salinity at the density of 15 individuals/tank with well aeration in triplicate. The water temperature recorded was 28 ± 1^0C and pH recorded was 8.0 ± 0.2. The shrimps were fed with different grades of experimental diets at *adlibitum* for 90 days i.e., starter feed for first 30 days, grower feed for second 30 days and finisher feed for third 30 days at the frequency of four times per day. The unfed remaining were collected prior to every feeding and dried in an oven at 80^0C. At the end of experiment, the animals were collected and weighed individually, sacrificed following the method of Maynard and Loosli (1962) and stored at -20^0C for further biochemical analysis. i. e., protein, carbohydrate, lipid and total phosphorus.

Growth responses

The growth performance parameters such as, food consumption (g), growth (g), SGR (%) and FCR were estimated using the formula described by Mohanty (1997 and 1999).

Nitrogen and phosphorus estimation

The methods for analysis of phosphorus and nitrogen in the diets, carcass and their consumption, loss and retention rates were estimated using the formula described by Watanabe *et al.* (1987b) and Takeuchi *et al.* (1989). The results obtained in the present study were subjected to relevant statistical analysis following the method described by Zar (1974).

RESULT

Table 1 Biochemical constituents of experimental diets CD1 to CD5

Biochemical Constituents	Experimental commercial diets				
	CD1	CD2	CD3	CD4	CD5
Protein (%)	35.66 ± 2.37	39.10 ± 4.13	39.93 ± 4.10	39.22 ± 3.84	40.50 ± 3.91
Carbohydrate (%)	16.96 ± 2.50	20.13 ± 2.95	19.12 ± 2.54	19.62 ± 2.96	18.35 ± 2.51
Lipid (%)	9.23 ± 1.14	10.77 ± 1.43	10.34 ± 1.37	9.8 ± 1.48	10.19 ± 1.64
Phosphorus (%)	0.918 ± 0.032	0.716 ± 0.026	0.871 ± 0.031	0.821 ± 0.028	0.932 ± 0.036
Energy Kj g^{-1}	14.40 ± 0.18	16.33 ± 0.17	16.16 ± 0.16	15.91± 0.14	16.12 ± 0.19
Energy P : E ratio	44.32	43.52	39.85	39.45	39.95

Each value is a mean of triplicate samples (± SD)

Experimental diets

The percentage biochemical composition of the commercial diets (CD1 to CD5) under investigation is shown in Table 1. The protein content ranged from 35.66 ± 2.37 to 40.50 ± 3.91%. The carbohydrate and lipid contents respectively ranged from 16.96 ± 2.50 to 20.13 ± 2.95% and from 9.23 ± 1.14 to 10.77 ± 1.43%. The phosphorus content varied between 0.716 ± 0.026 to 0.932 ± 0.036%. The energy level of the five commercial diets was in between 14.40 and 16.33 $KJ.g^{-1}$. The P: E ratio of the experimental diets varied between 39.45 and 44.32.

Table 2 Growth performance of *P. monodon* fed with commercial diets (CD1 to CD5) .

Performance parameters	Commercial diets				
	CD1	**CD2**	**CD3**	**CD4**	**CD5**
Initial weight (mg)	23.00 ± 0.46	20.00 ± 0.41	22.00 ± 0.36	22 ± 0.25	22.00 ± 0.35
Final weight (mg)	4058.0 ± 73.33	5538.0 ± 82.82	5468.0 ± 68.78	5284.0 ± 86.92	6069.0 ± 79.86
Consumption (g)	6.96 ± 0.25	8.81 ± 0.31	7.38 ± 0.27	7.40 ± 0.24	7.77 ± 0.26
FCR	1.70 ± 0.06^{a}	1.59 ± 0.04^{a}	1.35 ± 0.02^{ba}	1.40 ± 0.07^{bc}	1.28 ± 0.02^{bcd}
SGR (%)	5.75 ± 0.24^{a}	6.25 ± 0.32^{b}	6.13 ± 0.34^{cb}	$6.09\ 0.24^{bac}$	6.25 ± 0.20^{bc}

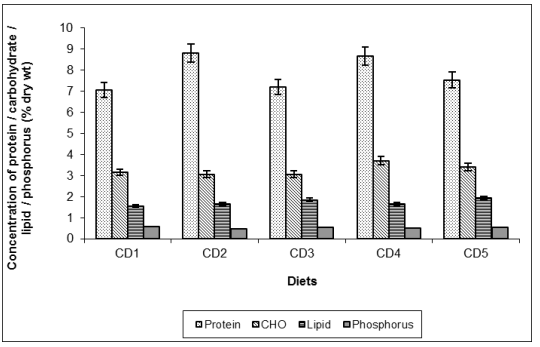
Each value is a mean of triplicate samples (± SD)

Rows with different alphabets are statistically significant (P<0.05: SNK test)

Growth performance

During the 90 days of the culture period, the growth performance parameters of *P. monodon* showed marked variation. The final weight of *P. monodon* fed on CD5 diet showed the maximum (6069.0 ± 79.86 mg) against the minimum weight (4058.00 ± 73.33 mg) recorded in CD1 diet fed group. Shrimps fed with other three commercial diets (CD2, CD3 and CD4) displayed more or less a uniform growth performance. Food consumption of *P. monodon* fed on commercial diets had similar trend except CD2 diet fed group, where a higher consumption was recorded (8.81 ± 0.31g). The specific growth rate was low in *P. monodon* fed on CD1 diet (5.75 ± 0.24 %) and high in CD2 and CD5 diets fed groups (6.25% each). The variation between SGR of all the dietary groups was statistically significant (P<0.05). Likewise, the FCR value of *P. monodon* fed on CD5 diet was better (1.28 ± 0.02); whereas, it was poor (1.70 ± 0.06) in CD1 diet fed shrimps and the variation between all the groups was statistically significant (P<0.05) (Table 2).

Fig. 1 Variation in carcass biochemical constituents of *P. monodon* fed with commercial diets (CD1 to CD5)



Shrimp whole body composition

The percentage dry weight biochemical composition of the whole body of shrimp fed with CD1 to CD5 diets are shown in Fig (1). Maximum carcass protein value was noticed in CD2 (8.81± 0.14%) diet fed shrimps against the minimum value of 7.06 ± 0.16% in CD1 diet fed group. The carbohydrate and lipid contents showed no marked variation and these values were ranged from 3.06 ± 0.06 to 3.71 ± 0.05% and 1.55 ± 0.03 to 1.93 ± 0.04% . Likewise the total phosphorus content did not vary much and it ranged from 0.481 ± 0.007 to 0.589 ± 0.004 %.

Table 3 Nitrogen metabolism of *P. monodon* fed with commercial diets (CD1 to CD5).

Experimental diets	Biomass		Shrimp nitrogen (% wet weight)	Nitrogen gain / retention (mg)	Nitrogen retention		
	Initial (g)	Final (g)			Amount of feed (g)	Feed nitrogen (mg/100 mg)	Nitrogen consumed (mg)
CD1	23.00 ± 0.46	4.06 ± 73.33	5.25 ± 0.13	212.63 ±[a] 6.80	6.90 ± 0.25	5.71 ± 0.18	397.42 ± 13.92[a]
CD2	20.00 ± 0.41	5.54 ± 82.82	6.56 ± 0.17	363.29±[b] 7.98	8.81 ± 0.31	6.26 ± 0.24	551.15 ± 18.79[b]
CD3	22.00 ± 0.36	5.47 ± 68.78	5.36 ± 0.14	293.08 ±[c] 5.89	7.38 ± 0.27	6.39 ± 0.20	471.51 ± 14.63[c]
CD4	22.00 ± 0.25	5.28 ± 86.92	6.44 ± 0.18	340.29±[d] 6.82	7.40 ± 0.24	6.28 ± 0.34	464.35 ± 12.76[d]
CD5	22.00 ± 0.35	6.07 ± 79.86	5.60 ± 0.15	339.86±[d] 5.86	7.77 ± 0.26	6.44 ±0.26	503.50 ± 16.62[e]

Each value is a mean of triplicate samples (± SD)

Values in a column with different alphabets are statistically significant (P<0.05: SNK test)

Table 4 Nitrogen retention / loss of *P. monodon* fed with commercial diets (CD1 to CD5)

Experimental diets	Nitrogen retention as % of feed	Total nitrogen loss (mg)	Nitrogen loss / g of shrimp produced (mg g^{-1})	Nitrogen loss / g of feed consumed (mg g^{-1})
CD1	53.81 ± 1.39	184.79± 4.42^{a}	45.54 ± 1.18^{a}	26.55 ± 0.48^{a}
CD2	66.08± 1.46	187.86± 5.68^{a}	33.92 ± 1.24^{b}	21.32 ± 0.62^{b}
CD3	62.37± 1.52	178.43± 4.74^{b}	32.63 ± 0.97^{b}	24.18 ± 0.73^{c}
CD4	72.52± 1.71	124.06± 2.86^{c}	23.48 ± 0.76^{c}	16.76 ± 0.31^{d}
CD5	67.26 ± 1.92	163.64± 3.24^{d}	26.96 ± 0.82^{d}	21.06 ± 0.36^{b}

Each value is a mean of triplicate samples (± SD)

Values in a column with different alphabets are statistically significant (P<0.05)

Table 5 Phosphorus metabolism of *P. monodon* fed with commercial diets (CD1 to CD5)

Experimental Diets	Biomass		Shrimp phosphorus (% wet weight)	Phosphorus gain / retention (mg)	Phosphorus retention		
	Initial (mg)	Final (mg)			Amount of feed (mg)	Feed phosphorus (mg/100 mg)	Phosphorus consumed (mg)
CD1	23.00 ± 0.46	406 ± 73.33	0.589± 0.015	23.910±^{a} 0.741	6.90 ± 0.25	0.918 ± 0.034	63.893± 1.406^{a}
CD2	20.00 ± 0.41	554 ± 82.82	0.482± 0.011	26.671±^{b} 0.872	8.81 ± 0.31	0.716 ± 0.030	63.106± 1.578^{a}
CD3	22.00 ± 0.36	547 ± 68.78	0.533± 0.016	29.139±^{c} 0.640	7.38 ± 0.27	0.871 ± 0.042	64.280± 1.221^{a}
CD4	22.00 ± 0.25	528 ± 86.92	0.524± 0.014	27.709±^{d} 0.859	7.40 ± 0.28	0.821 ± 0.045	60.754± 1.519^{b}
CD5	22.00 ± 0.35	607 ± 79.86	0.561± 0.013	34.059±^{e} 0.851	7.77 ± 0.26	0.932 ± 0.050	72.447± 1.817^{c}

Each value is a mean of triplicate samples (± SD)

Values in a column with different alphabets are statistically significant (P<0.05: SNK test)

Table 6 Phosphorus retention / loss of *P. monodon* fed with commercial diets (CD1 to CD5)

Experimental diets	Phosphorus retention as % of feed	Total phosphorus loss (mg)	Phosphorus loss / g of shrimp produced (mg g^{-1})	Phosphorus loss / g of feed consumed (mg g^{-1})
CD1	37.26 ± 0.90	39.983 ±^{a} 0.946	9.853 ±^{a} 0.256	5.745 ±^{a} 0.149
CD2	41.97 ± 0.98	36.437 ±^{b} 1.172	6.579 ±^{b} 0.144	4.136 ±^{a} 0.130
CD3	44.90 ± 1.02	35.141 ±^{b} 0.984	6.427 ±^{b} 0.138	4.762 ±^{bc} 0.136
CD4	45.04 ± 1.21	33.045 ±^{c}1.062	6.254 ±^{b} 0.140	4.466 ±^{bc} 0.142
CD5	46.74 ± 0.86	38.388 ±^{ad} 1.216	6.325 ±^{b} 0.170	4.941 ±^{c} 0.128

Each value is a mean of triplicate samples (± SD)

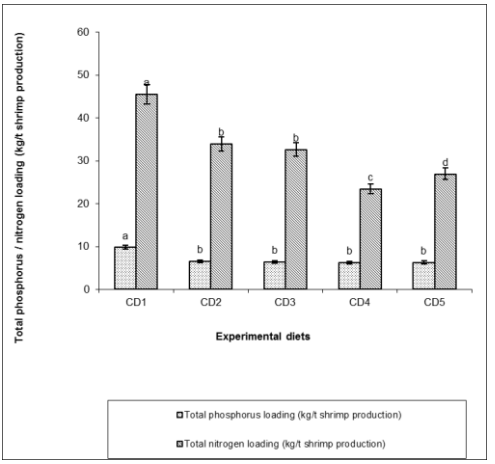
Values in a column with different alphabets are statistically significant (P<0.05: SNK test).

Retention of nitrogen and phosphorus

Nitrogen and phosphorus consumption, gain and retention of *P. monodon* fed on experimental diets were showed marked variations (Tables 3, 4, 5 and 6). Nitrogen consumption of *P. monodon* was high in CD2 (551.15 ± 18.79 mg) diet fed shrimps. The nitrogen gain of *P. monodon* also exhibited a maximum (363.29 ± 7.98 mg) in CD2 diet fed group and the variation between different diets fed groups was statistically significant ($P<0.05$) except CD4 and CD5 diet fed shrimps. Nitrogen retention of *P. monodon* as percentage of feed nitrogen was varied from 53.81 ± 1.39 to 72.52 ± 1.71%. The CD2 diet fed *P. monodon* showed the highest total nitrogen loss (187.86 ± 5.68%) against CD4 diet fed shrimps which were registered the low nitrogen loss (124.06 ± 2.86 %). The variation in total nitrogen loss of *P. monodon* fed CD1 to CD5 diets was statistically significant ($P<0.05$). The nitrogen loss per gram of shrimp produced was high in CD1diet fed *P.monodon* (45.54 ± 1.18 mg g^{-1}), followed by other diets fed shrimps. The nitrogen loss per gram of feed consumed was minimum (16.76 ± 0.31 mg g^{-1}) in CD4 diet fed group against the maximum of 26.55 ± 0.48 mg g^{-1} for CD1 diet fed shrimp.

Phosphorus consumption of *P. monodon* fed with experimental diets showed more or less similar values except CD5 diet fed group (72.44 ± 1.817 mg). The phosphorus gain / retention of *P. monodon* was also high (34.05 ± 0.85 mg) in CD5 diet fed group than other diets fed groups. The phosphorus retention as percentage of feed phosphorus had ranged from 37.26 ± 0.90 to 46.74 ± 0.86%. Total phosphorus loss was high (39.98 ± 0.94 mg) in CD1 diet fed group; whereas, it was in between 33.045 ± 1.06 and 38.388 ± 1.216 mg. Phosphorus loss per gram of shrimp produced was ranged from 6.254 ± 0.140 to 9.85 ± 0.256 mg g^{-1} and phosphorous loss/g of feed consumed was high in CD1 diet (5.74 ± 0.149 mg g^{-1}) fed group. More or less similar values were noticed in other diets fed groups.

Fig. 2 Total phosphorus and nitrogen loading (kg t^{-1}) shrimp production) of *P. monodon* fed with commercial diets (CD1 to CD5)



Each value is a mean of triplicate samples (± SD)
Values superscripted in a similar type of bar with different alphabets except CD1 diet, others are statistically significant ($P<0.05$: SNK test).

Nitrogen and phosphorus loading

Based upon nitrogen and phosphorus retention and shrimp production, total nitrogen and phosphorus loading were calculated for *P. monodon* fed on CD1 to CD5 diets. Total phosphorus loading was varied much in between CD1 to CD5 diets fed shrimps and it ranged from 6.25 to 9.85 kg t^{-1} shrimp production. Except CD1 diet, the variation in total phosphorus loading between other diets fed groups (CD2 to CD5) was not statistically significant ($P > 0.05$). The total nitrogen loading was also differed much and it ranged from 23.48 to 45.54 kg t^{-1} shrimp production. Multiple comparison of mean total nitrogen loading based on shrimp production indicated that, except between CD2 and CD3 diets, the variation between other diets was statistically significant ($P<0.05$) (Fig 2).

DISCUSSION

The growth response of *P. monodon* fed on CD1 diet was low when compared to those shrimp fed on CD2 to CD4 diets. Shrimps fed on CD5 diet showed the highest growth performance (6.069 mg). The FCR and SGR values recorded in CD5 diet fed shrimps were 1.28 and 6.24% respectively. Shrimps received CD1 to CD5 diets, the FCR and SGR values did not vary much and ranged from 1.28 to 1.70 and 5.75 to 6.25% respectively. The variation in growth responses of *P. monodon* fed on commercial diets may be attributed to the difference in concentration and availability of nutrients in the commercial diets. Watanabe *et al.* (1999) reported that the quality of commercial diets which are being used for carp production was largely fluctuated among the feed manufacturers.

The total nitrogen loss was higher (187.86 ± 5.68 mg) in CD2 diet fed group, but it was lower (124.06 ± 2.86 mg) in CD4 diet fed group. The higher nitrogen loss per gram shrimp produced (45.54 ± 1.18 mg g^{-1}) and also per gram of feed consumed (26.55 ± 0.73 mg g^{-1}) registered in *P. monodon* fed on CD1 diet may also attributed to the inclusion of different protein sources in commercial diets.

In the present study, the total nitrogen loading of *P. monodon* fed on CD1 to CD5 diets based on shrimp production was varied between 23.48 to 45.54 kg t^{-1} shrimp produced. This may be deviation/attribution to the nature of the commercial diets. This result is in consistent with the earlier report of Satoh *et al.* (1997) on rainbow trout. Watanabe and Ohta (1995) also reported that the protein quality of diets affected the nitrogen output into the water environment by altering the protein rate of carp and rainbow trout.

The phosphorus consumption and retention of *P. monodon* fed on CD1 to CD5 diets established a higher value (72.45± 1.817 and 34.06 ± 0.85 mg) in CD5 diet fed group, followed by other diets fed groups. Watanabe *et al.* (1999) reported that the difference in phosphorus absorption rate among the commercial diets might have resulted from the difference of phosphorus compounds supplemented in the diets in terms of mono or di-basic phosphorus. In the present study, *P. monodon* retained 37.42 to 47.01% of consumed phosphorus in the body. The variation in phosphorus retention as percentage of phosphorus consumed by *P. monodon* may be due to the variation in concentration of available phosphorus in diets. Chester *et al.* (1969) and Lall (1991) reported that 63 to 93% of absorbed phosphorus may retained in the fish body and the other parts might be excreted against into the digestive tract as observed in mammals or excreted through gills and urine.

Despite little variation in phosphorus retention as percentage of feed phosphorus, its loading based on shrimp production varied much in between shrimps fed on CD1 to CD5 diets (9.85 to 6.33 kg/t). Chester *et al.* (1969), Persson (1988), Takeuchi (1988), Weisman *et al.* (1988), Lall (1991), Roberto and Grahame (1996) were reported that observed difference in phosphorus loading might be originated from a loss of absorbed phosphorus through renal or gill excretion. In accordance with their results, in the present study, *P. monodon* utilized the absorbed phosphorus at varying range and resulted in significant variation ($P < 0.05$) in total phosphorus loading. This also implied that in addition to variation in dietary protein, the other macronutrients interactions including the interacting effect of dietary energy may also be responsible for the loading of dietary phosphorus in the environment. This said interaction effect also holds good for the loading of dietary nitrogen in the aquafarm environment. Further experiments will be needed to clarify the interacting effect of dietary

macronutrients and also dietary energy density and macronutrients on phosphorus and nitrogen loading in the shrimp culture ponds.

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