

Effect of Dietary Nutrient Sources on Nitrogen and Phosphorus Loading from Culture of Tiger Shrimp (*Penaeus monodon*)

Preetha VV^{1*}, Belayneh A^{1*}, Palavesam A^{2*}, Immanuel G² and Tadesse Z³

¹College of Natural & Computational Science, Haramaya University, Ethiopia

²Centre for Marine Science and Technology, M. S. University, India

³Ethiopian Institute of Agricultural Research, National Fishery & Aquaculture Research Centre, Ethiopia

Abstract

Effect of dietary nutrient source like, fishmeal (F1), soymeal (F2), casin (F3) and groundnut oilcake (F4) on nitrogen and phosphorus loading during culture of tiger shrimp, *Penaeus monodon* was assessed. Healthy shrimps (PL 25) with average body weight of 20.3 ± 0.3 mg were reared in 200 litre capacity FRP tanks containing 150 litre filtered seawater (20 ppt salinity) at the stocking density of 15 individuals/tank with well aeration in triplicate. Shrimps were fed with the experimental diets four times per day at ad libitum and the unfed remains were collected daily in the early hours and dried in an oven at 80°C. The growth of *P. monodon* was found to be greater (2.43 ± 0.07 g) with better FCR (1.71 ± 0.03) and SGR ($5.33 \pm 0.18\%$) on F1 diet. Total nitrogen losses were high in shrimps reared in F3 diet (73.34 ± 0.78 mg) whereas total phosphorus losses were high in F1 diet (37.279 ± 0.590 mg). Nitrogen loss per gram of shrimp produced and per gram of feed consumed showed maximum value (48.51 ± 0.49 and 23.21 ± 0.41 mg/g) in F3 diet fed group followed by other diets groups. Phosphorus loss per gram of shrimp produced and feed consumed were high in F1 diet fed shrimp. Total phosphorus loading (kg/t) based on shrimp production showed high value (15.34 kg/t shrimp produced) in F1 diet fed group, whereas it was low (1.087 kg/t shrimp produced) in F3 diet group. Total nitrogen loading based upon shrimp production showed maximum loading (48.5 kg/t shrimp produced) in F3 diet group and minimum value (20.2 kg/t shrimp produced) in F2 diet group. Therefore, in shrimp farming there is a need for consideration of availability of proper meal proportionate, nutritionally complete, cost-effective and aquaculture friendly green formulated feeds in order to achieve better productivity and aquaculture sustainability.

Keywords: Aquaculture; Dietary source; Nitrogen loading; Phosphorous loading; Tiger shrimp

Abbreviations: ANOVA: Analysis of variance; FCR: Feed Conversion Ratio; SGR: Specific growth rate; PL: Post larvae; FRP tanks: Fibreglass Reinforced Plastics; SNK: Student-Newman-Keuls multiple range test; CMC: Carboxy Methyl Cellulose; P:E: Protein : Energy ratio; SD: Standard Deviation

Introduction

Shrimp farming is one of the most outstanding commercial success stories in the history of Asian aquaculture, which produced more than 80% of the global cultured shrimp [1]. Out of 46.9 million tons of World inland aquaculture production, shrimp alone contribute 5% [2]. In most of the developing Asian countries, cultured shrimp production earning valuable foreign exchange and hence more and more areas are coming under shrimp farming including India. Success in shrimp farming is highly dependent in the availability of well balanced, nutritionally complete and cost-effective formulated feeds [3].

Along with the rapidly expanding shrimp farming, artificial feeds of different forms and composition have also been developed and widely used in every phase of culture from larval rearing to brood stock maturation and spawning [4]. The release of nitrogen and phosphorus from these artificial feeds to their habitat is of greater concern, because accumulation of these elements causes excessive algal bloom and eutrophication in that habitat [5-7]. Shrimp diets are prepared with higher level of protein and hence fish meal is added as the major ingredient like as the source of most dietary phosphorus. Fish meal, a main protein component in aquatic feeds, usually contains 2-4% phosphorus in the form of hydroxyl apatitate, which is almost unavailable to many cultivable species which lacks a stomach and devoid of gastric juice secretion [8-10]. Considering the limited supply of fish meal and also the need to reduce nitrogen and phosphorus excretion through retention in the

dietary nitrogen and phosphorus, plant derived ingredients are increasingly substituted for fish meal as protein source in salmon and trout diets [11,12].

In shrimp farming the tendency of over use of fish meal, rather than including both animal and plant protein ingredients, resulting in higher nitrogen and phosphorus excretion into the water system. That is why; one of the fundamental challenges facing the shrimp industry is to improve both environmental and economic performance by developing and implementing an integrated approach to reducing nitrogen and phosphorus wastes [5,6,13]. Hence this study was conducted to examine the effect of different dietary protein sources on nitrogen and phosphorus retention and loss in an outdoor shrimp culture system. In addition, the effect of different dietary treatments on growth performance, Carcass biochemical composition, FCR and SGR were investigated.

Materials and Methods

Collection of shrimp

For this study, post larvae (PL20) of tiger shrimp (*Penaeus mono-*

*Corresponding authors: Preetha VV, College of Natural & Computational Science, Haramaya University, P.O. Box 282, Ethiopia; Tel: 251- 915137322; E-mail: preethakanu@gmail.com

Anteneh Belayneh, College of Natural & Computational Science, Haramaya University, P.O. Box 282, Ethiopia, Tel: 251-911759139; E-mail: anthil2005@gmail.com

Received May 28, 2012; Accepted June 27, 2012; Published July 02, 2012

Citation: Preetha VV, Belayneh A, Palavesam A, Immanuel G, Tadesse Z (2012) Effect of Dietary Nutrient Sources on Nitrogen and Phosphorus Loading from Culture of Tiger Shrimp (*Penaeus monodon*). J Aquacult Res Dev 3:145 doi:10.4172/2155-9546.1000145

Copyright: © 2012 Preetha VV, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

don; Family: Penaeidae) were obtained from the Kanai Shrimp Hatchery, Kulasekharapattinam, Tuticorin District, Tamilnadu of India. The post larvae were transported to the laboratory in oxygenated bags and acclimatized to the ambient laboratory condition (salinity 20 ppt; temperature $28 \pm 1^\circ\text{C}$; pH 8.0 ± 0.2) in one ton capacity FRP tank for a period of 5 days and were then used for rearing experiment.

Experimental diets

Four experimental diets were prepared with varying protein source i.e. fish meal (F1), soya meal (F2), casein (F3) and groundnut oil cake (F4). In addition, other ingredients such as wheat flour, rice bran and cod liver oil were supplemented to maintain similar dietary contents. The additives like, vitamin and mineral mix and NaH_2PO_4 were also used. The proportions of various feed ingredients used for the preparation of experimental diets (F1, F2, F3 and F4) are shown in Table 1. The biochemical composition such as protein, carbohydrate, lipid and total phosphorus contents of all experimental diets were measured.

Estimation of protein: The protein reacts with copper sulphate to form a protein – copper complex. In the second step, this complex was allowed to reduce by the phosphomolybdolic-phosphotungstic acid complex. The reduced complex was blue in colour and measured colorimetrically at 660 nm [14].

Estimation of carbohydrates: The carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxy methyl furfural. This compound forms green colored products with anthrone reagent which can be measured at 630 nm [15].

Estimation of lipid: The quantitative determination of lipid by sulphophosphovanilin method depends on the reaction of total lipid extracted from the sample using chloroform methanol mixture with sulphuric acid. Phosphoric acid and vanillin to give a red colour complex. The intensity of red colour is directly proportional to the concentration of total lipid in the sample [16].

A solution of 0.5 m sodium carbonate buffer at pH 8.5 was used for the extraction of the total available phosphorus in tissue samples. The extracted phosphorus in the solution was estimated colorimetrically through spectrophotometer by developing blue colour of the chlorostannous indicator reduced to molybdic phosphoric acid [17].

The energy content of the experimental diets was also estimated in a Parr 1421 semi microbomb calorimeter (Parr instrument Co., Moline, USA). Energy content in the test diets was estimated indirectly by relating the level of macronutrients i.e. protein, carbohydrate and lipid contents of the diets with the energy equivalent of the respective nutrients

i.e. 5.65 kJ energy for 1.0 g protein, 4.15 kJ for 1.0 g carbohydrate and 9.40 kJ for 1.0 g lipid [18]. The caloric values were expressed as calories per gram (cal/gm) on dry weight basis. All the estimations were done in triplicate and mean values were calculated. Considering the protein and energy content of the particular diet, P:E ratio was calculated.

Feeding experiment

Healthy shrimps (PL 25) with average body weight of 20.3 ± 0.3 mg were reared in 200 litre capacity FRP tanks containing 150 litre filtered seawater (20 ppt salinity) at the stocking density of 15 individuals/tank with well aeration in triplicate, three tanks for each feed (a total of twelve tanks). Then one shrimp from each replicate were collected and the mean value was taken for each feed type. There were triplicate samples - The water temperature recorded was $28 \pm 1^\circ\text{C}$ and pH recorded was 8.0 ± 0.2 . During experimentation, the shrimps were fed with the experimental diets four times per day at ad libitum and the unfed remains were collected daily in the early hours and dried in an oven at 80°C . Fifty percentage of the tank water was changed daily so as to maintain proper water quality in all the system. The feeding experiment was conducted for 90 days. The growth of the shrimp was assessed by gravimetric method once in 10 days. At the end of the experiment, the animals were collected and weighed individually, sacrificed following the method of Maynard and Loosli [19] and stored at -20°C for further biochemical analysis.

Growth responses

The growth performance, biomass (g), Specific Growth Rate (SGR - %) and Feed Conversion Ratio (FCR) were estimated using the formula described by Mohanty [20,21]. Diet performance was evaluated by calculation of:

- Percent weight gain = $\frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$
- Specific growth rate = $100 \left(\frac{\ln \text{average of final weight} - \ln \text{average of initial weight}}{\text{number of culture days}} \right)$
- Feed conversion ratio (FCR) = $\frac{\text{total dry feed intake (g)}}{\text{wet weight gain (g)}}$
- Percent survival = $\frac{\text{final number of shrimp}}{\text{initial number of shrimp}} \times 100$

Nitrogen and phosphorus estimation

The amount of nitrogen and phosphorus in the diets (fishmeal (F1), soymeal (F2), casin (F3) and groundnut oilcake (F4)) and in the shrimp carcass; nitrogen and phosphorus consumption, gain, retention and loss rates were estimated following the formula described by [22-24].

Estimation of tissue phosphorus: A solution of 0.5 m sodium carbonate buffer at pH 8.5 was used for the extraction of the total available phosphorus in tissue samples. The extracted phosphorus in the solution was estimated colorimetrically through spectrophotometer by developing blue colour of the chlorostannous indicator reduced to molybdic phosphoric acid [17].

Nitrogen gain/retention = $\text{Tissue nitrogen} \times \text{Total biomass production (mg wet weight)}$

Nitrogen consumption = $\text{Feed nitrogen} \times \text{Total feed consumed (mg dry weight)}$

Total nitrogen loss = $\text{Nitrogen in feed} - \text{Nitrogen gain/retention (mg wet weight)}$

Constituents in grams	Experimental diets			
	F1	F2	F3	F4
Fish meal	74	-	-	-
Soya meal	-	74	-	-
Casein	-	-	36	-
Groundnut oil cake	-	-	-	74
Wheat flour	8	8	8	8
Rice bran	3	3	3	3
CMC	3	3	3	3
Tapioca powder	5	5	5	5
NaH_2PO_4	1	1	1	1
Oil	4	4	4	4
Vitamin	2	2	2	2
Cellulose	-	-	38	-

Table 1: Proportion of feed ingredients of prepared experimental diets (F1 to F4).

Nitrogen gain in fish (mg) = (Final wet wt x final nitrogen) – (Initial wet wt x initial nitrogen)

Nitrogen loss per g of shrimp produced (mg/g) = Total nitrogen loss/Total biomass

Nitrogen loss per g of feed consumed (mg/g) = Total nitrogen loss/ Total feed consumed

Phosphorus gain / retention = Tissue Phosphorus x Total biomass production (mg wet weight)

Phosphorus consumption = Feed Phosphorus x Total feed consumed (mg dry weight)

Total Phosphorus loss = Phosphorus in feed – Phosphorus gain/retention (mg wet weight)

Phosphorus gain in fish (mg) = (final wet wt x final Phosphorus) – (Initial wet wt x initial Phosphorus)

Phosphorus loss per g of shrimp produced (mg/g) = Total Phosphorus loss/ Total biomass

Phosphorus loss per g of feed consumed = Total Phosphorus loss/ Total feed consumed

Data analysis

The data obtained in this study were subjected to relevant statistical analysis following the method described by [25]. Individual weight was analysed using one-way ANOVA and Student-Newman-Keuls multiple range test (SNK). The parameters like, growth performance, biomass, SGR (%), FCR, Retention and loading of nitrogen and phosphorus were analysed in one-way ANOVA to determine if significant difference exist among the different diets fed groups. Results were considered statistically significant at the level of $P < 0.05$.

Results and Discussion

Biochemical composition of diets

The proximate compositions of the experimental diets are provided in Table 2. The protein content varied from 29.68 ± 0.356 to $34.06 \pm 0.545\%$. The carbohydrate and lipid contents ranged from 15.06 ± 0.27 to $22.03 \pm 0.446\%$ and from 4.91 ± 0.078 to $13.20 \pm 0.211\%$ respectively. High phosphorus content was recorded in fish meal ($1.284 \pm 0.017\%$) diet and low phosphorus value was measured in casein added diet ($0.0942 \pm 0.001\%$).

Growth performance of shrimps

The growth performance of *Penaeus monodon* (Fabricius, 1998) (Family Penaeidae) fed with four test diets (i.e. fish meal, soya meal, casein and groundnut oil cake) for a period of 90 days showed marked

Biochemical constituents per 100 mg	F1	F2	F3	F4
Protein %	34.06 ± 0.545	31.12 ± 0.467	31.16 ± 0.560	29.68 ± 0.356
Carbohydrate %	18.58 ± 0.260	22.03 ± 0.446	15.77 ± 0.092	15.06 ± 0.271
Lipid %	13.20 ± 0.211	9.09 ± 0.127	4.91 ± 0.078	8.40 ± 0.136
Phosphorus	1.284 ± 0.017	0.343 ± 0.005	0.0942 ± 0.001	0.2742 ± 0.004
P : E	46.71	45.73	31.45	41.88
Energy (Cal/g)	3936.3	3526.9	2876.5	3090.6

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

Table 2: Proportion of biochemical constituents of prepared experimental diets (F1 to F4) per 100 mg of each ingredients.

Performance parameters	Experimental diets			
	F1	F2	F3	F4
Initial wt (mg)	20.0 ± 0.45	20.5 ± 0.40	20.6 ± 0.50	20.0 ± 0.46
Final wt. (mg)	2430 ± 0.073	1810 ± 0.062	1510 ± 0.051	1490 ± 0.042
Consumption (mg)	4160 ± 0.125	3310 ± 0.010	3160 ± 0.110	2860 ± 0.100
FCR	1.71 ± 0.03^d	1.83 ± 0.04^{bc}	2.09 ± 0.04^a	1.92 ± 0.06^b
SGR (%)	5.33 ± 0.176^a	5.01 ± 0.145^a	4.81 ± 0.130^b	4.79 ± 0.140^b

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

Note: Values in a row with different alphabets are statistically significant ($P < 0.05$; SNK test)

Table 3: Performance parameters of *P. monodon* fed experimental diets (F1 to F4).

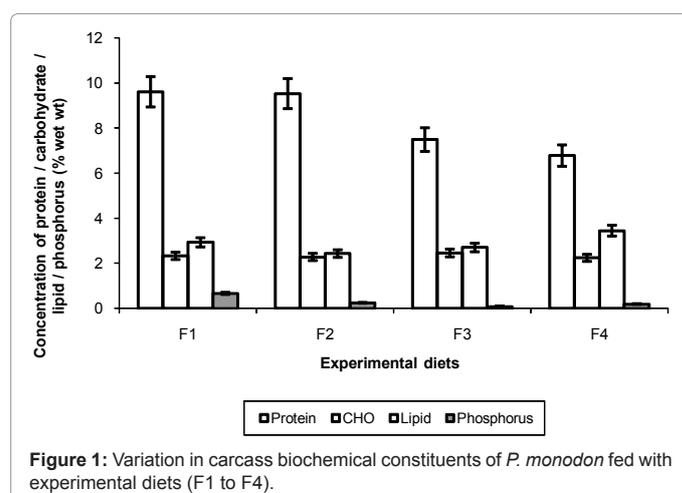


Figure 1: Variation in carcass biochemical constituents of *P. monodon* fed with experimental diets (F1 to F4).

variation (Table 3). The final weight of *P. monodon* fed on F1 diet was maximum (2.43 ± 0.073 g), but in the other diets (F2 – F4) fed groups ranged between 1.49 ± 0.04 and 1.81 ± 0.06 g. The food consumption was positively correlated with the final weight gain. Better feed conversion ratio (1.71 ± 0.03) was registered in fish meal (F1) supplemented diet fed *P. monodon*. The result is in consistence with the fact that to ensure better performance in black tiger shrimp, animal matter is essential [26]. The variation in FCR value of *P. monodon* which were fed on various experimental diets was statistically significant ($P < 0.05$) except between F1 and F2 diets fed groups. The trend noticed for the SGR of *P. monodon* was more or less similar to that of FCR values. The variation in SGR of *P. monodon* fed on test diets was statistically significant ($P < 0.05$) except between F1 and F2 diets fed groups. This variation in growth response of *P. monodon* fed on F1 to F4 diets may be attributed by the variation in source of nutrient in the test diets.

Various studies revealed that variation in growth response of shrimp could be attributed by the variation in source of nutrient in the test diets [4, 27]. Of course there are many studies indicating that the replacement of one meal with the other didn't show significance differences on the growth performance of shrimp [3,28]. This will encourage looking for an appropriate feed that will result optimum growth by considering effluent management in shrimp farming.

Carcass biochemical composition

The percentage biochemical composition of a shrimp carcass fed with F1 to F4 diets are shown in Figure 1. Protein content of shrimps fed with F1 and F2 diets showed more or less similar values (9.61 ± 0.15 and $9.53 \pm 0.17\%$) and a low value was noticed in F4 diet fed group. Carcass carbohydrate content showed no marked variation between dietary groups and it ranged from 2.24 ± 0.03 to $2.46 \pm 0.04\%$. Maximum lipid content was noticed in F4 diet fed group and in other groups

Feed	Biomass		Shrimp nitrogen (% dry wt)	Nitrogen gain / retention (mg)	Nitrogen consumption		
	Initial (mg)	Final (mg)			Amount of feed (mg)	Feed nitrogen (mg/100 mg)	Nitrogen consumed (mg)
1	20.0 ± 0.45	2430 ± 0.073	7.15 ± 0.164	173.82 ± 2.78 ^a	4160 ± 0.125	5.45 ± 0.145	226.56 ± 7.15
2	20.5 ± 0.40	1810 ± 0.062	7.09 ± 0.167	128.26 ± 1.86 ^b	3310 ± 0.010	4.98 ± 0.174	164.80 ± 4.16
3	20.6 ± 0.50	1510 ± 0.051	5.57 ± 0.460	84.22 ± 1.34 ^c	3160 ± 0.110	4.99 ± 0.160	157.56 ± 3.85
4	20.0 ± 0.46	1490 ± 0.042	5.04 ± 0.580	75.10 ± 1.27 ^d	2860 ± 0.100	4.75 ± 0.164	135.87 ± 3.65

Each value is a mean (X ± SD) of triplicate samples
 Note: Values in a row with different alphabets are statistically significant
 (P < 0.05; SNK test)

Table 4: Nitrogen metabolism of *P. monodon* fed on experimental diets (F1 to F4).

Feed	Biomass		Shrimp phosphorus (% wet wt)	Phosphorus gain / retention (mg)	Phosphorus retention		
	Initial (mg)	Final (mg)			Amount of feed (mg)	Feed phosphorus (mg/100 mg)	Phosphorus consumed (mg)
1	20.0 ± 0.45	2430 ± 0.073	0.664 ± 0.021	16.135 ± 0.419 ^a	4160 ± 0.125	1.284 ± 0.017	53.414 ± 1.175
2	20.5 ± 0.40	1810 ± 0.062	0.2473 ± 0.008	4.476 ± 0.125 ^b	3310 ± 0.010	0.343 ± 0.055	11.340 ± 0.295
3	20.6 ± 0.50	1510 ± 0.051	0.089 ± 0.002	1.336 ± 0.032 ^c	3160 ± 0.110	0.094 ± 0.001	2.977 ± 0.017
4	20.0 ± 0.46	1490 ± 0.042	0.195 ± 0.005	2.908 ± 0.076 ^d	2860 ± 0.100	0.274 ± 0.004	7.842 ± 0.227

Each value is a mean (X ± SD) of triplicate samples
 Note: Values in a row with different alphabets are statistically significant
 (P < 0.05; SNK test)

Table 5: Phosphate metabolism of *P. monodon* fed on experimental diets (F1 to F4).

(F1 to F3) the values showed no variations. But phosphorus content varied much from F1 to F4 diet fed groups. Maximum phosphorus content was recorded in F1 diet fed group (0.664 ± 0.008) and a minimum value in F3 diet fed group (0.089 ± 0.001).

The factors which affect the growth performance of fishes also exert their influence on carcass biochemical composition. The little variation noticed in the carcass biochemical composition of *P. monodon* may be attributed to the variation in source of nutrient in the test diets. Erfanullah and Jafri [29] reported that the effect of different carbohydrate sources on fingerlings of *L. rohita*, a higher amount of carcass carbohydrate content was recorded, when fed on diet containing 30% sucrose and 40% protein. Changes in whole body composition in tilapia *Oreochromis mossambicus* with respect to variation in dietary protein was also reported by Al Hafedh [30]. Nematipour et al. [31] also reported the marked variation in whole body dry matter, lipid and protein content.

The source and composition of feed stuffs also bring about significant variation in digestibility as reported in *Oreochromis aureus* and *O. niloticus* [32]. The variation noticed in carcass biochemical composition of *P. monodon* may be due to the difference in the digestibility co-efficient of the given nutrient sources which are compatible with the result reported by [33].

Retention and loading nitrogen and phosphorus

Nitrogen and phosphorus consumption, gain and retention of *P. monodon* showed a decreasing trend with respect to the addition of alternative dietary protein ingredients in the diets and it ranged between 226.56 ± 7.15 to 135.87 ± 3.65 mg. The higher nitrogen consumption (226.56 ± 7.15 mg) was noticed in F1 diet fed *P. monodon* (Table 4). Nitrogen gain of *P. monodon* was also showed a similar trend with that of nitrogen consumption and it ranged between 173.82 ± 2.78 mg in F1 diet fed group to 75.10 ± 1.27 mg in F4 diet fed shrimps (Table 4). The variation in nitrogen gain of *P. monodon* fed test diets was statistically significant (P < 0.05). Nitrogen retention of *P. monodon* as percentage of feed nitrogen was varied from 53.03 ± 0.85% in F3 diet fed group to 77.07 ± 1.37% in F2 diet fed group and the variation between F1 and F2,

F3 and F4 diets fed groups was not statistically significant (P > 0.05). A similar report was also made earlier by [16,17].

The results on phosphorus consumption of *P. monodon* was high in fish meal protein diet (F1) fed group (53.41 ± 1.18 mg) and low in casein protein diet (F3) fed group (2.98 ± 0.089 mg) (Table 5). The phosphorus gain/retention of *P. monodon* was high in F1 diet fed group and low in (1.336 ± 0.032 mg) F3 diet received shrimps and the variation between the test diets was statistically significant (P < 0.05). Phosphorus retention as percent of feed phosphorus was high (45.05 ± 0.41%) in *P. monodon* received diet with casin (F3) (Table 7). SNK test indicated that the variation in phosphorus retention as percentage of feed phosphorus was statistically significant except between F2 and F4 diet fed group.

The total nitrogen loss was also established a marked variation and it was higher in F3 and F4 diets. Maximum nitrogen loss (73.34 ± 0.78 mg) was measured in F3 diet fed group against the minimum of 36.54 ± 0.67 mg obtained in F2 diet fed group and the variation between them was statistically significant (P < 0.05). The nitrogen loss per gram of shrimp produced was the highest (48.51 ± 0.49 mg/g) in F3 diet fed group and lowest (20.20 ± 0.38 mg/g) in F2 diet fed shrimps (Table 6). Multiple comparison of mean total nitrogen loss per gram of shrimp produced indicated that the variation between them was statistically significant (P < 0.05). But nitrogen loss per gram of feed consumed showed much variation and it ranged between 11.04 ± 0.17 and 23.21 ± 0.41 mg/g (Table 6). In this respect the use of F2 diet (soyameal) along with the F1 diet (fishmeal) is more encouraged for high shrimp production and sustainable aquaculture.

The total phosphorus loss was high in F1 (37.28 ± 0.590 mg) and low in F3 (1.641 ± 0.042 mg) diets fed groups and the variation was statistically significant (P < 0.05) (Table 6). Phosphorus loss per gram of shrimp produced (mg/g) was also high in F1 diet fed group and low in F3 and F4 diets fed groups. Phosphorus loss per gram of feed produced showed a similar trend with that of total phosphorus loss. Phosphorus loss/g of shrimp produced ranged between 8.961 (F1) to 0.519 (F3) mg/g (Table 7). Multiple comparison of mean total phosphorus loss per

Feed	Nitrogen retention as % of feed	Total nitrogen loss (mg)	Nitrogen loss / g of shrimp produced (mg/g)	Nitrogen loss / g of feed consumed (mg/g)
1	76.62 ± 1.23 ^a	52.74 ± 1.20 ^a	21.69 ± 0.35 ^a	12.69 ± 0.18
2	77.07 ± 1.37 ^a	36.54 ± 0.67 ^b	20.20 ± 0.38 ^b	11.04 ± 0.17
3	53.03 ± 0.85 ^b	73.34 ± 0.78 ^c	48.51 ± 0.49 ^c	23.21 ± 0.41
4	54.04 ± 0.78 ^b	60.77 ± 0.84 ^d	40.79 ± 0.52 ^d	21.24 ± 0.37

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

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

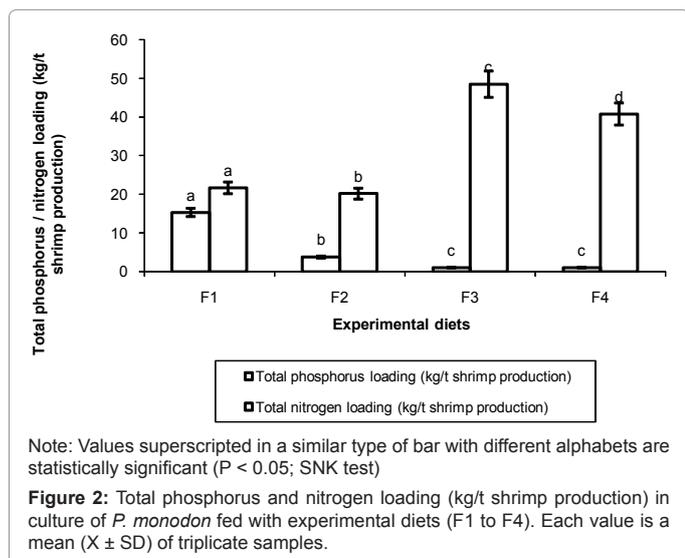
Table 6: Nitrogen loss in *P. monodon* fed with experimental diets (F1 to F4).

Feed	Phosphorus retention as % of feed	Total phosphorus loss (mg)	Phosphorus loss / g of shrimp produced (mg/g)	Phosphorus loss / g of feed consumed (mg/g)
1	29.78 ± 0.42 ^a	37.279 ± 0.590 ^a	15.341 ± 0.245 ^a	8.961 ± 0.134
2	39.45 ± 0.37 ^a	6.864 ± 0.087 ^b	3.792 ± 0.057 ^b	2.074 ± 0.031
3	45.05 ± 0.41 ^b	1.641 ± 0.042 ^c	1.087 ± 0.013 ^c	0.519 ± 0.007
4	37.49 ± 0.32 ^c	4.934 ± 0.088 ^d	1.087 ± 0.015 ^c	1.725 ± 0.034

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

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

Table 7: Phosphorus loss in *P. monodon* fed with experimental diets (F1 to F4).



Note: Values superscripted in a similar type of bar with different alphabets are statistically significant (P < 0.05; SNK test)

Figure 2: Total phosphorus and nitrogen loading (kg/t shrimp production) in culture of *P. monodon* fed with experimental diets (F1 to F4). Each value is a mean (X ± SD) of triplicate samples.

gram of shrimp produced revealed significant difference except between F3 and F4 diets fed groups.

Considering the nitrogen and phosphorus retention, the total nitrogen and phosphorus loading was calculated in *P. monodon* fed on F1 to F4 diets. Total phosphorus loading was high in F1 (15.34 kg/t) diet and low in F3 and F4 (1.09 kg/t each) diets and the variation between was statistically significant (P < 0.05) (Figure 2). On the other hand, the total nitrogen loading showed a significant difference (P < 0.05), and maximum loading was noticed in F3 (48.5 kg/t shrimp produced) and F4 (40.79 kg/t shrimp produced) diets fed groups and minimum loading in F1 (21.69 kg/t shrimp produced) and F2 (20.2 kg/t shrimp produced) diets fed groups (Figure 2). It was also revealed that a large portion of input nitrogen and phosphorus into shrimp ponds as feed is not converted to shrimp biomass, but is released into the environment [34-36] that could result in algal blooming and eutrophication. On the other hand, with respect to variation in source of protein, the phospho-

rus loading varied much being the maximum in F1 diet fed shrimps and it was comparatively high (P < 0.05) when compared to F2 – F4 diets fed groups. This result indicated that the inclusion of fish meal as the major protein substitute in F1 diet resulted in increased accumulation of total phosphorus. A similar observation was also made by Jahan et al. [37] in carp culture as the result of increase in concentration of dietary fish meal. Hence even at low dietary protein level of 30 to 34% inclusion of fishmeal as the major protein source could result in accumulation of more amount of phosphorus in shrimp culture system. As stated by [13] of the feed input at a food conversion ratio of 20 only 24% of the nitrogen and 13% of the phosphorus was incorporated into the shrimp harvested, whilst the remainder was retained in the pond and ultimately exported to the surrounding environment.

Conclusion

In shrimp farming there is a need for consideration of appropriate meal proportionate in order to achieve better productivity and habitat management. For example, an inclusion of fish meal as the major protein substitute in F1 diet resulted in high growth with better FCR and FGR, high nitrogen gain and consumption and increased accumulation of total phosphorus whereas resulted in high phosphorus loading to the aquaculture. The F3 meal also resulted in minimum phosphorus loading, whereas maximum loss per gram of shrimp produced and high nitrogen loading based upon shrimp production to the shrimp culture system. Therefore, there is a need to minimize F3 meal from the shrimp meal proportion. The results showed that the need for using different meals such as, soymeal, casin and groundnut oilcake relatively having less environmental impact rather than focusing only on fish meal, with proper proportion by considering the degree of nitrogen and phosphorus retention and loss in shrimp culture. By doing so, one can achieve better productivity and maintain sustainability of the aquaculture. In general, in shrimp farming there is a need for consideration of availability of proper meal proportionate, nutritionally complete, cost-effective and aquaculture friendly green formulated feeds in order to achieve better productivity and aquaculture sustainability.

References

1. Prasad G (1996) The world aquaculture production. The present and future role of India. Seafood Export J 7: 13-16.
2. Kungvankij P, Tiro LB, Pudadera BJ, Potestas IO, Chua TE (1985) An Improved Traditional Shrimp Culture Technique for Increasing Pond Yield. Technology Series No.1 FAO Aquaculture Newsletter.
3. Myrna N, Bautista-Teruel, Perla SE, Timothy PW (2003) Utilization of feed pea, *Pisum sativum*, meal as a protein source in practical diets for juvenile tiger shrimp, *Penaeus monodon*. Aquaculture 225: 121-131.
4. Boonyaratpalin M (1996) Nutritional requirements of commercially important shrimps in the tropics.
5. Federico Paez-Osuna (2001) The environmental impact of shrimp aquaculture: causes, effects, and mitigating alternatives. Environmental Management 28: 131-140.
6. Christopher J, Nigel P, Peter JT, Michele B (2003) Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. Aquaculture 218: 397-411.
7. Ling TY, Buda D, Nyanti L, Norhadi I, Emang JJJ (2010) Water quality and loading of pollutants from shrimp ponds during harvesting. J Environ Sci Eng 4: 13-18.
8. Ogino C, Takeuchi L, Takeda H, Watanabe T (1979) Availability of dietary phosphorus in carp and rainbow trout. Nippon Suisan Gakk 45: 1527-1532.
9. Yone Y, Toshima N (1979) The utilization of phosphorus in fish meal by carp and Black Sea bream. Nippon Suisan Gakk 45: 753-756.
10. Satoh S, Voranop V, Takeuchi T, Watanabe T (1997) Availability of phosphorus in various phosphates to carp and rainbow trout determined by a simple fractionation method. Fisheries Sci 63: 297-300.

11. Hardy RW (1996) Alternate protein sources for salmon and trout diets. Anim Feed Sci Technol 59: 71-80.
12. Vielma J, Marinen T, Kholm P, Koskela J (2000) Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. Aquaculture 183: 349-362.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193: 265-275.
14. Briggs MRP, Fvng-Smith SJ (1994) A nutrient budget of some intensive marine shrimp ponds in Thailand. Aquac Res 25: 789-811.
15. Seifter S, Dayton S, Novic B, Muntwyler E (1950) The estimation of glycogen with the anthrone reagent. Arch Biochem 25: 191-200.
16. Folch J, Lees I, Sloane Staneley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226: 497-509.
17. Olsen SR, Cole CV, Watanabe FS, Dean AL (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Dep. Agriculture 939.
18. Phillips AM (1969) Nutrition, Digestion, and Energy Utilization. Fish Physiol 1: 391-432.
19. Maynard AL, Loosli KC (1962) Animal nutrition, McGrawhill, Newyork, 553.
20. Mohanty P (1997) Studies on scientific management of semi-intensive culture of black tiger shrimp, *Penaeus monodon* at Balasore-Bhadrak coast of Orissa state (India). PhD Thesis submitted to Orissa University.
21. Mohanty P (1999) Growth performance of *Penaeus monodon* at different stocking density. J Inland Fish Soc 31: 44-49.
22. Watanabe T, Takeuchi T, Satoh S, Wang K, Ida T, et al. (1987) Development of practical carp for reduction of total nitrogen loading on water environment. Nippon Suisan Gakk 53: 2217-2225.
23. Watanabe T, Takeuchi T, Satoh S (1987) Development of low protein high energy diets for practical carp culture with special reference to reduction of total nitrogen excretion. Nippon Suisan Gakk 53: 1413-1423.
24. Takeuchi T, Watanabe T, Satoh S, Martino R.C, Ida T, et al. (1989) Suitable levels of protein and energy in practical carp diets. Nippon Suisan Gakk 55: 521-527.
25. Zar JH (1999) Biostatistical Analysis (4thedn), Prentice Hall, New Jersey, 663.
26. Menasveta P, Yu Y (2002) Replacement of fish meal with meat and bone meal or poultry by product on growth performance of black tiger prawn, *P. monodon*. Research report No. 22. Asia Regional Office of the national Associations Inc., Causeway Bay. Hong Kong, 3.
27. Akiyama DM, Coelho SR, Lawrence AL, Robinson EH (1988) Apparent digestibility of feedstuffs by the marine shrimp *Penaeus vannamei* Boone. Bull Jap Soc Fish 55: 91-98.
28. Cruz-Suárez LE, Nieto-López M, Guajardo-Barbosa C, Tapia-Salazar M, Scholz U, et al. (2007) Replacement of fish meal with poultry by-product meal in practical diets for *Litopenaeus vannamei*, and digestibility of the tested ingredients and diets. Aquaculture 272: 466-476.
29. Erfanullah R, Jafri AK (1995) Growth response of fingerling Indian major carp, *Labeo rohita* (Ham) to various sources of dietary carbohydrate. J Aqua Trop 10: 287-296.
30. Al Hafedh YS (1999) Effect of dietary protein on growth and body composition of Nile tilapia (*Oreochromis niloticus* L.). Aquac Res 30: 385-393.
31. Nematipour GR, Brown ML, Galtin DM (1992) Effects of dietary carbohydrate: Lipid ratio on growth and body composition of hybrid striped bass. J World Aquacult Soc 23: 128-132.
32. Degami G, Yehuda Y (1999) Digestibility of protein sources in feed for *Oreochromis aureus* x *O. nilotica*. Ind J Fish 46: 33-39.
33. Plakas SM, Katamaya T (1981) Apparent digestibility of amino acids from three regions of the gastrointestinal tract of the carp (*Cyprinus carpio*) after ingestion of a protein and a corresponding free amino acid diet. Aquaculture 24: 309-314.
34. Jackson C, Preston N, Thompson PJ, Burford M (2003) Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. Aquaculture 218: 397-411.
35. Xia LZ, Yangans LZ, Yan MC (2004) Nitrogen and phosphorus cycling in shrimp ponds and the measures for sustainable management. Environ Geoch Health 26: 245-251.
36. Paez-Osuna F, Ruiz-Fernandez AC (2005) Environmental load of nitrogen and phosphorus from extensive, semi-intensive and intensive shrimp farms in the Gulf of California eco-region. Bulletin Environ Contam Toxicol 74: 681-688.
37. Jahan PA, Watanabe TA, Satoh SH, Kiron VI (2000) Effect of dietary fish meal levels on environmental phosphorus loading from carp culture. Fish Sci 66: 204-210.