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Effect of crude protein levels and biofloc density on growth performance of Pacific shrimp *Litopenaeus vannamei*

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Abstract
Feeding trails were conducted to evaluate the effects of crude protein level in the feed and biofloc density on feed utilization and growth performance of Pacific shrimp *Litopenaeus vannamei* in an integrated biofloc system. The experimental diets were formulated with 25,35 and 45% crude protein with a combination of 15 and 30 ml/lit biofloc density and feeding trails were conducted for 90 days. From the results obtained in the present study, experimental diets having a combination of 35% crude protein with 15 ml/lit biofloc density substantially improved the feed utilization rates and simultaneously induced the highest and best growth potentials in *L. vannamei*. Experimental diets formulated with 25 and 45% crude protein, though also fared well, but unable to excel the rates of feed utilization and growth indices compared to 35% CP containing diet. So the combination of 35% protein feed with 15 ml/lit biofloc density capable of promoting the supplemental food source and thus excellent growth rates in *L. vannamei* culture operation.

Keywords: *L. vannamei*, bioflocs, molassess, crude protein

1. Introduction
Shrimp production has rapidly expanded with the development and intensification of aquaculture system; however, this successful industry has been threatened by several diseases caused by opportunistic pathogens [1, 2]. In recent times, several studies have focused on the design and development of a sources of alternative production systems for the culture of candidate species in aquaculture operation. Such systems have the main objectives on reducing the use of water through culture operation and to meet the several demands of increased stocking densities [3, 4]. Several approaches have been developed by Aquaculturists to prevent diseases in the culture operation with the advent of closed water recirculation systems and best management of aquaculture practices, but these methods were identified to be rather not effective. Therefore, it is very essential to explore other alternative technologies, which are more effective and economical, includes application of Biofloc technology, which plays a key role in disease prevention, maintenance of water quality and recycle the faeces and leftover food into a microbial protein that can be utilized as a feed supplement by shrimp [5, 6, 7]. Biofloc technology is based on microbial manipulation with in the aquaculture system i.e. Carbon: Nitrogen (C:N) ratio in feed has been shown promising results [8, 9]. This Biofloc technology was generated by supplementation of external Carbon sources in the feed and contributes for increased productivity rates in the candidate species of culture [10-13]. Therefore the present investigation is aimed to determine the optimum biofloc density and crude protein levels for the evaluation of experimental diet for Pacific shrimp *Litopenaeus vannamei*, through monitoring of growth performance parameters by using sugarcane molasses as external carbon source for the production of Bioflocs.

2. Materials and Methods
The present set of experiments were carried out in shrimp culture units located in Ramayapatnam (Latitude 15^0 02^1 55^{11} N; Longitude 80^0 02^1 50^{11} E), Prakasam district of Andhra Pradesh, India. Penaeid shrimp *L. vannamei* of 1.52 ± 0.07 g of uniform size were obtained from local Aqua farms and were tested for diseases occurrence and finally pathogen specific free shrimp were selected for experimentation.

The selected shrimp were acclimatized in experimental tanks with a specific Salinity of 10 ± 0.5 ppt. The completely randomized design method with two independent variables were used in the present study, the first variable was Crude Protein (CP) level in the diet (25, 35 & 45% Protein levels) and the second was Floc volume or density (FD) in tank reared medium (15 and 30 ml/lit)

2.1 The Experimental groups are follows

Group-I: Control group fed with Commercial diet obtained from local market; Group-II: CP 25% with 15 ml/lit FD; Group-III: CP 25% with 30 ml/lit FD; Group-IV: CP 35% with 15 ml/lit FD; Group-V: CP 35% with 30 ml/lit FD; Group-VI: CP 45% with 15 ml/lit FD; Group-VII: CP 45% with 30 ml/lit FD.

The present experimental setup consisted of 1000 lit plastic open tanks containing 80% water volume with 10 ppt Salinity water and continues aeration was provided with the help of all compressors. The experimental diets with 25, 35 and 45% crude protein (CP) containing diets were formulated and the mixing of ingredients was presented in Table.1. Ingredients including Fish meal, Prawn meal, Squilla meal (Animal protein sources), Soyabean meal and wheat flour (Plant sources) were selected for formulation of experimental diets and stored for subsequent use in experimentation [14].

2.2 Preparation of Biofloc

Sugarcane molasses was selected as a source for carbon and known to contain 36% carbon, 53% carbohydrate, 24% moisture content was incubated for 2 days in warm water at 40^0 C and the same was added to the culture medium in the ratio of 1:3 Molasses: Water. To stimulate Nitrogen loading in an aquaculture system NH_4Cl, KH_2PO_4 and Na_2HPO_4 were added to biofloc culture tank @ 96, 31 and 64 mg/lit, respectively [15]. The ratio between sugarcane molasses and feed to reach desired Carbon: Nitrogen (C:N) ratio was calculated based on assuming 50% nitrogen from feed eaten by the shrimp excreting in to the water environment [8]. On the above basis the formula of the ratio in weight between, the Carbon source and feed can be given as follows by arriving precisely at C:N (15:1).

$$\frac{\Delta CH}{\Delta F} = \frac{(CN \times \%\ P\ (F) \times \%\ N\ (P)) - \%\ CF}{\%\ C_{CH}}$$

Where

ΔCH: Weight of Carbon Source

ΔF: Weight of the Feed

CN: C: N ratio need to be required

% P(F): Protein Content in Feed

% N(P): Nitrogen Content in Protein (15.5%)

% CF: Carbon Content in the Feed (50%)

% C_{CH}: Carbon content in the Carbon source.

Floc volume and density were calculated as follow by adopting the method of Isnansetyo & Kurniatuti[16] as follows:

$$\text{Floc volume:} = \frac{\text{Floc density in culture media} \times \text{Volume of the water in culture tank}}{\text{Floc density in the growing tank}}$$

The floc density in the culture media was controlled at a constant level, if the floc was high siphoning of water was adopted, while in floc density was decreased, floc from growing tank was added to the culture media to a desired level.

Growth Parameters were calculated by adopting the following formulae;

$$\text{Survival rate (\%)} = \frac{\text{Total number of Live shrimp Stocked - Total number of shrimp dead}}{\text{Total Number of shrimp stocked}}$$

Weight gain (g);

Weight of the Shrimp (g) - Weight of the Shrimp (g)

At the end of the Experiment At the start of the Experiment

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total amount of Feed consumed (Kgs)}}{\text{Total Biomass of Shrimp (Kgs) gained during Experiment}}$$

$$\text{Average daily growth rates (ADGR)} \frac{\text{Weight of the Shrimp (g) At the end of the Expt} - \text{Weight of the Shrimp (g) at the start of the Expt}}{\text{Total number of days of Experiment.}}$$

$$\text{Specific growth rates (SGR):} \frac{\text{Log weight of the Shrimp (g) At the end of the Experiment} - \text{Log Weight of the shrimp (g) at the Start of the Experiment}}{\text{Total number of days of Experiment.}}$$

The data obtained was analyzed statistically through Microsoft excel. The difference in variants between treatments was determined according to one-way ANOVA using SPSS.

3. Results and Discussion

In the present investigation an attempt has been made to study the feed utilization and growth performance studies in pacific white shrimp *Litopenaeus vannamei* after subjected to different combinations of Feed protein levels with variable Floc density concentrations in feeding trails. In the present study three crude protein levels were selected i.e. 25, 35 and 45% with Floc density combination of 15 and 30 ml/lit. The ingredient composition of Experimental feeds was presented

in Table.1. All the experimental feeds were formulated by selecting locally available feed ingredients including animal proteins represented by Fish, Prawn and Squilla meal, whereas plant protein substances represented by Soybean meal and wheat flour along with basic components for the formulation of experimental feeds. The proximate composition of the experimental feeds formulated in the present investigation was presented in Table.2. Parameters like Organic matter, Ash, NFE, Crude Protein, Crude Lipid, Crude fibre, Moisture and Gross energy levels were estimated and all the values obtained were found to be in the normal range. The organic matter content was found to be around 82% in all the experimental diets. The Ash content was found to be in the range of 17%. The NFE values obtained were showing higher values in 25% CP containing experimental feeds (38%) compared to 45% CP containing feeds (19%). The crude protein values obtained were found to be 25, 35 and 45% in the experimental feeds. Crude lipid values falls in and around 6%, whereas crude fibre values are around 4%. The moisture content was found to be around 8%. Which as normal for all shrimp feeds. The gross energy levels were found to be 400 kcal/100 g in all the experimental feeds formulated in the present investigation. Growth performance and Feed utilization details of *L.vannamei* under feeding trails with different protein and Floc densities and presented in Table.3. In each feeding trail 300 Nos of Juveniles were stocked. The percent survival rates were found to be more than 90% in all the feeding trail experiments. The final weights recorded in the present study were 12.74 g with control tank fed with commercial feed, among the three crude protein levels selected highest levels of growth weights recorded with 35% with CP and 15 & 30 ml/lit floc densities and minimum recorded with 25% CP with 15 & 30 ml/lit Floc densities, whereas intermediate range recorded with 45% CP with 15 & 30 ml/lit floc densities. Similar kind of trends were also obtained pertaining to weight gain with *L.vannamei*. Daily Growth rates were also found to be recorded significantly higher with 35% CP feed (0.19 g/day) compared to 25 & 45% CP containing feed. The best Feed conversion ratio of 1.75 recorded with 35% CP feed with 15 ml/lit Floc density compared to other 25 & 45% CP feed with 15 & 30 ml/lit Floc density. The Specific growth rates were observed to be best with 35% CP feed with 15 ml/lit floc density (1.20) compared to other experimental feeds. The Feed efficiency values obtained were also found to be significantly higher with 35% CP feed with 15 ml/lit Floc density (57%) compared to other experimental feeds. The productivity values recorded to be highest with 35% CP feed with 15 ml/lit Floc density (5.33 Kgs) compared to other experimental feeds containing 25 & 45 CP with 15 & 30 ml/lit Floc densities. The trends obtained for growth performance and feed utilization studies clearly demonstrates that, 35% CP containing feed appears to be best in inducing growth potentials comparing to 25 & 45% CP feeds with 15 & 30 ml/lit Floc densities.

Growth performance and feed utilization of the shrimp were significantly affected by combinations of protein levels and biofloc density (Table.3). Creating a new environment and ecosystem for culturing of shrimps and became a commercial venture in several cases of affected areas either due to diseases or other problems. In recent years, new management practices have been studied for production of shrimps. These practices accentuate reduced water exchange and focus on the optimization of culture conditions and improvements in several aspects of shrimp culture including biosecurity. Beneficial role of BioFloc Technology (BFT) system in penaeid shrimp has been well documented [17, 18]. BFT improving water quality by addition of extra Carbon consequential encouraged nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification [19]. Recently it was also reported that the use of biofloc as a dietary supplement had enhanced growth rate of *L.vannamei* [20, 21, 22]. In the present study, dietary supplementation of biofloc density @ of 15 & 30 ml/lit with a combination of Crude protein 35% significantly ($P< 0.05$) enhanced growth, FCR, ADGR & SGR in shrimp *L. vannamei* compared to 25 and 45% CP containing experimental diets. It has been documented that bioflocs are the rich source of many bioactive compounds such as carotenoids, chlorophyll, phytosterols, bromophenols, amino sugars [22] and antibacterial compounds [9]. Thus, the enhanced growth performance of shrimp fed with biofloc containing diet in the present study might be explained by a bioactive or probiotic microbial compounds, such as *Bacillus* or *Lactobacillus* sps present in the biofloc on the other hand the CP levels 25% or 45% with biofloc supplementation in the density of 15 or 30 ml/lit did not result in proportionate enhancement of growth rates and FCR. In between the two i.e. 25 or 45% CP containing diets, the 45% CP containing diets showed better results in terms of induction of growth potentials compared to 25% CP diets in the present study. From the results obtained in the present study it is very clear that, increasing CP content in the diets with a constant biofloc density, has showed maximum growth rates in the 35% CP diets and attained peak stage, but though there was an increase in CP in beyond 35%, no significant higher growth rates were observed. Several authors reported that diets supplemented with bioflocs recorded significantly higher growth rates at 10 and 15%, but no significant difference at 20 and 30% dietary biofloc inclusion [20]. The results obtained in the present study were also in agreement with earlier reports. Wang [23] and Anand *et al*. [24], who reported that increment of dietary Probiotics in shrimp does not proportionally increase growth performance in shrimp. Moreover, reduction in growth was recorded at high level of microbial supplementation [25, 26]. As microbial products at higher levels tend to reduce feed palatability and digestibility [26]. Correia *et al*. [27] and Yun *et al*. [28] demonstrated that for *L.vannamei* reared in biofloc systems. The dietary protein level could be with no adverse effect on shrimp growth and body composition. But Xu & Pan [29] reported that, a reduction of dietary protein level (35% for 25%) in biofloc system improves the biofloc proximate composition and the extra cellular enzyme activities (amylase), will probably contributes to shrimp growth. The data obtained in the present study suggest that high protein (45%) with low protein (30%) feed in an integrated biofloc system can reduce shrimp production costs because of reduced use of crude protein. Shrimp growth variables are highly sensitive to protein quality, especially to adequate supply of essential amino acids [30]. Brito *et al*. [31] recorded increased crude protein levels in shrimp (in whole bodies) reared in integrated biofloc systems.

The present study may be concluded that the use of an integrated biofloc system i.e. Crude protein and biofloc density can improve feed utilization and growth performance of *L.vannamei*. The results also showed that feed dietary protein at 35% with 15 ml/lit biofloc density substantially improves the growth potentials, its suggests the use of

integrated biofloc systems is a sustainable strategy for supporting shrimp growth. Further studies are needed to fully understand the potential role of interaction between crude protein vs biofloc density for promoting shrimp nutrition when provided as a supplemental food source in integrated biofloc systems.

Table 1: Composition of Experimental diets with different Protein and Floc density levels

Feed Ingredient	Protein Percentage		
	25	**35**	**45**
Fish meal	8	12	15
Prawn meal	8	12	15
Squilla meal	8	12	15
Soy bean meal	32	25	21
Wheat Flour	30	25	20
Vitamin mixture	1	1	1
Mineral mixture	1	1	1
Cod liver oil	6	6	6
Cholesterol	0.5	0.5	0.5
Ascorbic acid	1	1	1
Choline chloride	1	1	1
Chromic oxide	0.5	0.5	0.5
Agar agar	3	3	3
	100	100	100

Table 2: Proximate Composition of Experimental diets

Parameter	**Control**	**Protein Percentage**					
		25		**35**		**45**	
		Biofloc Density (ml/lit)		**Biofloc Density (ml/lit)**		**Biofloc Density (ml/lit)**	
		15	**30**	**15**	**30**	**15**	**30**
Organic matter	82.74±2.82	83.11±2.74	82.73±2.74	82.77±2.49	83.14±2.19	81.88±2.74	83.05±2.79
Ash	17.26±0.55	16.89±0.62	17.27±0.64	17.23±0.58	16.86±0.64	18.12±0.58	16.95±0.61
NFE	34.44	38.14	37.72	28.34	28.5	18.86	18.95
Crude protein	30.12±1.24	26.77±1.22	26.94±1.28	36.18±12	36.35±1.34	44.77±1.35	45.84±1.42
Crude Lipid	6.38±0.14	6.42±0.15	6.18±0.15	6.29±0.14	6.34±0.15	6.38±0.14	6.41±0.16
Crude Fiber	3.87±0.11	3.74±0.12	3.77±0.12	3.82±0.13	3.74±0.14	3.72±0.13	3.69±0.13
Moisture	7.93±0.49	8.04±0.55	8.12±0.58	8.14±0.59	8.21±0.62	8.15±0.45	8.16±0.52
Grass Energy (Kcal/100 g)	386	382	379	393	396	403	410

Values are Mean ± SD of six individual observations.
Organic Matter : 100 – Ash
NFE : 100 – (CP + CL + CF + Ash + Moisture)
Gross Energy : (CP x 5.6) + (CL + 9.44) + (NFE x 4.1) kcals/100 g

Table 3: Growth performance and Feed Utilization details of *L.vannamei* under different Feeding trails with different Protein and Floc density

Parameter	**Control**	Protein Percentage					
		25		**35**		**45**	
		Biofloc Density (ml/lit)		**Biofloc Density (ml/lit)**		**Biofloc Density (ml/lit)**	
		15	**30**	**15**	**30**	**15**	**30**
Animals Stocked	300	300	300	300	300	300	300
Percent Survival	90	93	92	98	97	95	95
Final weight (g)	12.74	14.19±0.21	14.85±0.21	18.13±0.28	17.83±0.28	16.11±0.24	14.54±0.22
Weight gain (g)	11.22[a] (+738)	12.67±0.18[a] (+853)	13.33±0.19[a] (+877)	16.61±0.23[a] (+1092)	16.31±0.25[a] (+1073)	14.59±0.24[a] (+960)	13.02±0.22[a] (+857)
Average daily growth rate (g)	0.13 PDC	0.14[c] (+7.7)	0.15[a] (+15.38)	0.19[a] (+46.15)	0.18[a] (+38.46)	0.17[a] (+30.76)	0.15[a] (+15.38)
Feed conversion ratio (FCR)	2.84 PDC	2.53[b] (-11)	2.48[b] (-13)	1.75[a] (-38)	1.94[a] (-32)	2.17[a] (-24)	2.52[b] (-11)
Specific growth rate (SGR)	0.97 PDC	1.08[b] (+11)	1.106[b] (+14)	1.2[a] (+24)	1.19[a] (+23)	1.17[a] (+21)	1.12[b] (+16)
Feed efficiency (FE) (%)	35 PDC	39[b] (+11)	40[b] (+14)	57[a] (+63)	52[a] (+49)	46[a] (+31)	40[b] (+14)
Productivity (Kgs)	3.44 PDC	3.96[b] (+15)	4.1[b] (+19)	5.33[a] (+55)	5.19[a] (+51)	4.48[a] (+42)	4.37[a] (+27)

Shrimp with Average Weight of 1.52 ± 0.07 g were stocked.
Values presented in parenthesis are Percent Change over their respective Control
All values are Statistically Significant at [a]$P< 0.05$; [b]$P< 0.01$; [c]NS: Not Significant

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