



Efficacy of Vegetable Wastes, Probiotics and Bioflocs on the Growth Performance of White Shrimp *Litopenaeus vannamei*

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ABSTRACT

The present study was conducted to investigate in monitoring the effect of vegetable wastes, Probiotics and Bioflocs on the growth and performance parameters of Pacific white shrimp *Litopenaeus vannamei* in 90 day feeding trail experiments. Due to the addition of vegetable wastes, Probiotics and Bioflocs into the culture operation significantly increased the growth potentials in shrimp. The vegetables are known to contain biomolecules including lipids, carbohydrates, proteins, minerals, vitamins and phytonutrients which were forming as rich sources to shrimp growth and maintenance. In the present study the vegetable wastes were substituting the fishmeal contents in the feed formulation and also successfully catering the needs of the growth, molting, metabolic requirements of shrimp. Due to the addition of probiotics and bioflocs into the culture operation also facilitates not only the maintenance of hygienic aquatic environment and also increases the production of heterotrophic bacterial growth and large quantities of planktonic forms, which in turn will be useful as supplementary feeding materials and subsequently increase not only the growth rates and also productivity. The growth parameters such as weight gain, specific growth rate, feed conversion efficiency, protein efficiency rates were significantly ($P < 0.05$) higher in probiotic and biofloc added feeds compared to vegetable waste formulated feed. Feed conversion ratios were shown to be decreased in probiotic (2.15) and biofloc (1.93) added feeds compared to vegetable waste (2.54) formulated feeds in the present investigation. The energy utilization parameters, such as feeding, absorption, conversion, excretory and metabolic rates were significantly ($P < 0.05$) higher compared to vegetable waste incorporated control feed. So, vegetable wastes may be considered as an ideal replacement for fishmeal in the formulation of shrimp feed along with the addition of probiotics and bioflocs for successful promotion of growth potentials for shrimp *L. vannamei* culture operation.

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Introduction

The rapid worldwide expansion of aquaculture to meet up with the global demand of fish and shrimp is an indication of an impending pressure on the aqua-feed industries. Since many fisheries are over-fished, aquaculture is recognized as the only feasible option to meet the increasing demands for aquatic feeds¹. Feed is one of the major input in aquaculture production but the feed technology is one of the least developed sector of aquaculture particularly in Asian countries, including developing countries of the world. In aquaculture more than half the investment comprising 50-70% of the total operating costs goes into feeds as they contribute an essential factor for enhancing aquaculture production. Fishmeal as a raw material is the choice in aquaculture due to its high quality protein with balanced amino acid profile^{2,3}. Though the production rates of fishmeal has been relatively stable in the last two decades but the increasing demand could not be matched in the present world due to increased aquaculture activity. Moreover, the cost of fishmeal is increasing which renders the use of alternative protein source which are local, cheap and available in lumpsum quantities to make up for the shortage of fishmeal and secure the stable supply for the formulation of

aquaculture commercial feeds for several candidate species. Several researchers conducted series of works done on the nutritional value of many alternative feed ingredients to supplement the dietary fishmeal-based diets. Several authors also suggested the alternative protein sources derived from Animal and Plant sources can be used to formulate nutritious aquaculture feed⁴. The identification and development of alternative feed ingredients than can replace fishmeal is recognized as an international research priority. Feed stuffs of vegetable origin as a whole are lower in protein when compared to those of animal origin, nevertheless among all the plant protein sources tested for most crustaceans, soybean meal has been found to be the most superior on account of its high protein content and essential amino acid profile^{5,6}. In order to reduce the escalating cost of aqua feed and make aquaculture sustainable in the long run intensive research is being focused on alternative and more sustainable protein sources for use within compounded aquafeeds. The utility of plant protein as partial replacement for the more expensive animal protein fractions has been examined but results show great variations in the degree of success, which inordinately depend on the species and types of ingredients used. Non conventional ingredients that are capable of partially or

completely substituting the fishmeal requirements in the formulation of feeds for shrimp, should be more abundant and less expensive. Quite a large number of vegetables are discarded as wastes, which includes, Cabbage, Green leaves, Carrot, Beet root are available in bulk quantities in local vegetable markets. The vegetable wastes available in the local markets were incorporated as a non-conventional ingredients in shrimp feeds would therefore be a lucrative proposition. It would be most pertinent to carry out further enrichment of these substances as well along with these vegetable waste in order to increase their nutritive and digestibility values. The present study was conducted to evaluate the suitable level of replacement of fishmeal with vegetable waste along with the addition of both Probiotics and Bioflocs and to assess the growth promotion, nutritional and energy utilization parameters of Pacific White shrimp *Litopenaeus vannamei*.

Materials & Methods

The present set of experiments were conducted at shrimp culture units located in Ramayapatnam (Latitude 15° 02' 55" N; Longitude 80° 02' 50" E) Prakasam District of Andhra Pradesh, India. The experiments were randomly designed which includes three feeding trail groups

Group I : Control group without Probiotics and Bioflocs

Group II : Control group with Probiotic fermented

Group III : Control group Probiotic fermented and Bioflocs added.

Penaeid shrimp *Litopenaeus vannamei* (0.79 ± 0.03 g) of uniform size, pathogen free were collected from local aquaculture farms and were acclimatized in field station in large cement tanks (2 × 1.5 × 1.0 mts). The cement tanks were provided with soil collected from aquaculture ponds in order to provide earthen pond environment. The cement tanks were filled with water drawn from treated storage tank. The hydrobiological parameters were maintained as Salinity (10 ± 1 ppt), Temperature (27 ± 1°C), pH (7.5 ± 0.1) constantly throughout the experimentation. All the experimental tanks were intermittently aerated with the help of Air compressors. Feeding was done ad libitum twice a day both in the morning and evening at 6.00 AM and 6.00 PM. The experimental feeding trails were conducted for a period of 90 days.

The vegetable wastes including Cabbage (*Beassica oleracea*), Carrots (*Daucus carota*), Potatoes (*Solanum tuberosum*), Tomato (*Solanum esculentum*), Cauliflower (*Brassica oleracea*) and Beans (*Phaseolus vulgaris*) were collected from vegetable market processed daily by using a steam of water to remove any dirt, vigorously blended in a power operated blender (1400 rpm), dried in the sun, milled into small particles with a locally manufactured feed grinder and then preserved into airtight plastic containers. Depending on the Dry matter content fresh vegetable waste, rice polish was added as an absorbent during blending to facilitate quick drying.

Preparation of Biofloc

Sugarcane molasses was selected as a source for Carbon and known to contains 36% Carbon, 53% Carbohydrate, 24% moisture content was incubated for 2 days in warm water at 40°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 NH₄Cl, KH₂PO₄ and Na₂HPO₄ were added to each tank @ 96, 31 and 64 mg/lit, respectively⁷. 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 environment⁸. On the above basis

the formula of the ratio in weight between, the carbon source and feed can be given as follows

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

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

Carbon content was determined by adopting the method of Walkley and Black⁹. Total Ammonia Nitrogen (TAN) concentration and other water quality parameters were measured with the procedures according to APHA¹⁰. Growth parameters including, average body weights, average body growth rates, specific growth rates, feed conversion ratio, protein efficiency ratio, feed efficiency ratio and productivity rates were monitored and tabulated. All the above parameters were calculated by adopting the following Formulae.

Survival Percentage (%):

$$\frac{\text{Total number of live shrimp}}{\text{Total number shrimps stocked}} \times 100$$

Weight gain (g):

Weight of the Shrimp (g) - Weight of the Shrimp (g)
At the end of the expt. at the start of the expt.

Feed Conversion Ratio (FCR):

$$\frac{\text{Total amount of Feed consumed (Kgs)}}{\text{Total Biomass of Shrimp (Kgs)}}$$

Average Daily Growth Rates (ADGR):

Weight of the Shrimp (g) - Weight of the Shrimp (g)
At the end of the Expt. at the start of the Expt.

Total number of days of Experiment.

Specific Growth Rates (SGR):

[Log weight of the shrimp (g) - Log weight of the shrimp (g)]
At the end of the Expt. at the start of the Expt.

----- × 100

Total number of days of experiment

(Log W₂ - Log W₁)

----- × 100

T

Where:

W₁ : Weight of the shrimp at the start of the Experiment

W₂ : Weight of the shrimp at the end of the Experiment

T : Total number of days of Experiment.

Biofloc volume (FV)

V Floc / V collection

Where

VFloc : Biofloc Volume (ml)

V collection : Collected Sample Volume (ml)

Similarly feeding trails were conducted for calculating food utilization parameters, such as feeding rate, mean absorption, mean conversion and metabolic rate. The energy content of whole prawn, feeds, faeces, and exuvia was measured using Oxygen Bomb Calorimeter. The energy budget was calculated using the equation (C = (P + E) + R + F + U) derived by Petruszewicz & Macfadyen¹¹; where, C is the energy consumed in food; P. is the conversion or growth; R, the material lost as heat due to metabolism; F, the energy lost through faeces; U, the energy lost in ammonia excretion

and E, the energy lost through exuvia. The daily excretion of ammonia by the prawn was estimated after feeding as per the phenol hypochloride method of Solorzano¹². The energy loss occurring by ammonia excretion was calculated using the ammonia calorific quotient, 1 mg NH₃ : 5.9 cal¹³. The food energy consumed was measured as the difference between the energy content of food offered and that of the uneaten food. The quantity of absorbed food energy was calculated by subtracting F from C. conversion or growth is the sum of energy channelled to somatic growth (P) and exuvia (E), Following the estimations of C, F, U and P, the metabolism (R= Respiration) was calculated by dividing the respective amount of energy by initial live weight of the prawn per unit time in days.

$$\text{Feeding Rate} = \frac{\text{Mean Food Consumption (k cal day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean Absorption} = \text{Mean Food Consumption (k cal day)} - \text{Mean Food Excreted as Faeces (k cal day)}$$

$$\text{Absorption Rate} = \frac{\text{Mean Absorption (k cal day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean Conversion} = \text{Mean weight gain (k cal day)} - \text{Mean exuvial weight (k cal day)}$$

$$\text{Conversion rate, P} = \frac{\text{Mean Conversion (k cal day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{NH}_3 \text{ Excretion rate, U} = \frac{\text{Mean NH}_3 \text{ Excretion (k cal day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Metabolic Rate, R} = \text{Absorption rate (k cal g day)} - \text{Conversion rate (k cal g day)} - \text{NH}_3 \text{ excretion rate (k cal g day)}$$

$$\text{Metabolic Rate, R} = \text{Absorption rate (k cal / g / day)} - \text{Conversion rate (k cal / g / day)} + \text{NH}_3 \text{ Excretion rate (k cal / g / day)}$$

Probiotic Feed Preparation:

Probiotic supplemented feeds were prepared as described by Naresh¹⁴. Probiotic bacterial species *Bacillus licheniformis* and *Lactobacillus rhamnorus* were obtained and maintained in the nutrient broth and were harvested by centrifuging at 10,000 rpm for 10 minutes subsequently washed with phosphate buffer, finally re-suspended in phosphate buffer saline (pH 7.4). These resuspended bacteria were mixed uniformly to the vegetable waste along with several ingredients. The Probiotic blended feed prepared was dried at 40⁰ C and packed in air tight polythene covers and stored in Refrigerator for further use. The Probiotic blended feed with *L. rhamnorus* and *B. licheniformis* (@ 10 billion cfu / kg) feed were prepared once in seven days.

The composition of experimental feeds formulated was presented in Table. 1.

Statistical Analysis

One way analysis of variance (ANOVA; SPSS, 13.0) was used to determine whether significant variation between the treatments existed. Difference between means were determined and compared by DMRT test. All the tests used a significance level of P < 0. 05. Data are reported as means ± standard deviations.

Results and Discussion

In the present investigation an attempt was made monitor the effect of incorporation of vegetable wastes in the feed in

place of fishmeal and incorporation of Probiotics and Bioflocs on the growth potentials of Pacific white Shrimp *Litopenaeus vannamei*. The proximate composition of different experimental feeds formulated was presented in Table. 2. Among the three feeds formulated, i.e. Control feed formulated with vegetable waste along with several ingredients, another feed formulated with vegetable waste fermented with probiotic *B. licheniformis* and *L. rhamnorus*, and lastly the above feed with Biofloc added in to the culture medium. All the above feeds formulated were found to be isoproteic and isoenergetic . The crude protein content was found to be around 40%, where as crude lipid and crude fibre contents were around 7 and 4%, respectively. The moisture content was around 8% and the gross digestible energy levels were found 430 k cal/100 g. Several authors indicated that the protein requirement for both *Penaeid* and *Palaemonid* shrimp ranges between 35-40% and 7-8% of lipid as optimum in the diet. Similarly the Carbohydrate requirement also ranges from 25- 40% as optimum in the diet. In accordance with the above reports several researchers, in the present study, the protein, carbohydrate and lipids supplemented to be optimum and ranges around 40% protein, 25% carbohydrate and 7% lipid for the shrimp *L.vannamei*. In the present study, the reaching percentage of formulated feed pellets also studied and the reaching percentage was found to be 10-13% in 6 hrs period. The leaching percentage was relatively 13% in the control diet compared to experimental diets formulated either with the inclusion of Probiotics or Biofloc was found to be 10%. It clearly suggests that due to addition of either Probiotics or Bioflocs enhances the feed stability or reduces the reaching rate. Immanuel et al¹⁵ reported that the inclusion of fishmeal in formulated feeds and the increase of fishmeal proportion have decreased the water stability. It has been reported that the stability of pelletized feed is being influenced by different factors such as composition of the feed, nature of ingredients, moisture content, types of processing¹⁶. Generally, the compounded pelletized feeds are considered as high -energy nutritive packages, they should be stable in water with a low rate of disintegration such that they will be remain available for consumption by shrimp for a longer periods, without the loss of nutrients through leaching. So, feeds with high stability were considered as one of the major advantageous, as they posses high nutrient value and also easily being consumed by shrimp. In the present study, both Probiotic and Bioflocs added feed group feeding trails recorded greater growth rates compared to control diet, which possess only vegetable waste. There were significant differences in growth patterns (P<0.05) on variations between experimental and control feed fed groups. The performance of shrimp *L.vannamei* after fed with control and experimental diets after 90 days of feeding trails was presented in Table.3.

In each experimental treatment for feeding trails shrimp of equal size 0.79 ± 0.03 g pre acclimatized shrimp were selected and stocked into respective feeding trail group. The percent survival values recorded 88% with control diet when compared to 95% with both the experimental diets, which was considered to be significant(P<0.05). During these feeding trail experiments, the shrimp showed normal behaviour without any remarkable observations. Therefore, the Probiotic or Bioflocs added feeds fed prawns were resulted in significantly higher survival rates when compared to control group.

Table 1. Composition of Experimental feeds

Ingredient	Experimental diets		
	Control	Probiotic Fermented	Probiotic Fermented + Biofloc Added
Vegetable Waste	0	25	25
Fishmeal	30	10	10
Shrimp meal	15	10	10
Yeast meal	5	5	5
Maize	10	10	10
Wheat flour	10	10	10
Soybean	10	10	10
Groundnut oil cake	5	5	5
Cod liver oil	4	4	4
Vegetable oil	4	4	4
Gelatin	5	5	5
Vitamin C	0.5	0.5	0.5
Mineral mixture	1	1	1
Vitamin mixture	0.5	0.5	0.5
Total	100	100	100

Table 2. Proximate composition of different experimental diets (% DM basis)

Parameter	Experimental diets		
	Control	Probiotic Fermented	Probiotic Fermented + Biofloc Added
Organic matter	82.78 ± 2.74	82.94 ± 2.49	83.04 ± 2.41
Ash	16.14 ± 0.95	16.49 ± 1.21	16.77 ± 0.95
Crude Protein	39.85 ± 1.49	40.05 ± 1.22	40.11 ± 1.18
Crude Lipid	7.03 ± 0.25	6.89 ± 0.24	6.94 ± 0.25
Crude Fiber	4.15 ± 0.15	4.26 ± 0.18	4.29 ± 0.19
Nitrogen Free Extract	30.13 ± 1.12	31.42 ± 1.14	31.49 ± 1.42
Moisture	8.12 ± 0.72	8.01 ± 0.64	8.71 ± 0.62
Gross Energy (kcal/100g)	430	436	437

Organic Matter : 100 – Ash NFE : 100 – (CP+CL+CF+Ash+Moisture)

Gross Energy : (CPx5.6) + (CLx9.44) + (CFx4.1) + (NFEx4.1) kcal/100 g

Table 3. Performance details & Energy Utilization parameters of *L. vannamei* under different feeding trails

Parameter	Experimental diets		
	Control	Probiotic Fermented	Probiotic Fermented + Biofloc Added
Shrimp stocked (nos)	1000	1000	1000
Percent Survival (%)	88	95	95
Final Weight (g)	21.98 ± 0.34 ^a PDC + 28	28.18 ± 0.29 ^b	31.33 ± 0.32 ^{bc} + 43
Weight Gain (g)	21.19 ± 0.31 ^a PDC + 24	27.39 ± 0.34 ^b	30.54 ± 0.31 ^{bc} + 39
Daily Growth Rate (DGR) (g)	0.235 ^a PDC + 29	0.304 ^b	0.339 ^{bc} + 44
Specific Growth Rate (SGR)	2.49 ^a PDC + 5	2.61 ^a	2.66 ^a + 7
Protein Efficiency Ratio (PER)	5.38 ^a PDC + 50	8.05 ^b	8.18 ^b + 52
Feed Conversion Ratio (FCR)	2.54 ^a PDC - 15	2.15 ^b	1.93 ^b - 24
Feed Conversion Efficiency (FCE) (%)	39 ^a PDC + 20	46.74 ^b	52.26 ^{bc} + 34
Feed Efficiency Ratio (FER)	0.394 ^a PDC + 18	0.465 ^b	0.518 ^{bc} + 31
Harvest Size (g)	21.45 ± 0.35 ^a PDC + 31	28.04 ± 0.34 ^b	31.35 ± 0.36 ^{bc} + 46
Productivity (kgs)	18.88 ^a PDC + 41	26.64 ^b	29.79 ^a + 59
Feeding Rate	0.581 ± 0.025 ^a PDC + 44	0.834 ± 0.032 ^b	0.928 ± 0.034 ^{bc} + 60
Absorption Rate	0.424 ± 0.021 ^a PDC + 42	0.602 ± 0.024 ^b	0.654 ± 0.032 ^{bc} + 54
Conversion Rate	0.384 ± 0.021 ^a PDC + 23	0.474 ± 0.024 ^b	0.548 ± 0.028 ^{bc} + 43
Ammonia Excretion Rate	0.041 ± 0.004 ^a PDC + 63	0.067 ± 0.004 ^b	0.069 ± 0.004 ^b + 68
Metabolic Rate	0.313 ± 0.014 ^a PDC + 49	0.467 ± 0.021 ^b	0.494 ± 0.022 ^{bc} + 58

All Values are Mean ± SD of six individual observations

Values presented in parenthesis are Percent Change over their respective Control

Values with different superscripts are significantly different from each other @ P < 0.05.

The results obtained in the present investigation are in accordance with the earlier reports that Probiotics i.e Lactic acid bacteria added diets significantly enhanced the percent survival rates from 92 to 98% in *P. indicus* juveniles¹⁷, in *P. monodon* due to the addition of Probiotics i.e. *Bacillus sps* supplemented diets^{18,19}, in freshwater prawn *M. rosenbergii* fed with *L. acidophilus* and *L. sporogenes* diets recorded 100% survival²⁰. Soon after the completion of 90 day feeding trail experimentation, the final weights and also average harvest sizes were recorded and tabulated. The final weights recorded to be 21.98, 28.18 and 31.33 g for control, Probiotic and Biofloc added groups, respectively. Similarly the weight gain values were found to be 21.19, 27.39 and 30.54 g for control, Probiotic and Biofloc added groups, respectively. One way analysis of variance revealed that the variation in weight gain of shrimp *L. vannamei* between control and experimental diets were statistically significant at $P < 0.05$. Similar results on significant improvement in weight gain were reported in white shrimp *L. vannamei*^{21,22} using Probiotic bacteria includes *B. licheniformis* and *L. rhamnorus* and bioflocs using Sugarcane molasses, Rice flour, Wheat flour as carbon sources. Similar kind of observations were also reported in freshwater prawn *M. rosenbergii* by using *L. sporogenes*²⁰, in *M. amazonicum* by supplementation of diets with *Saccharomyces cerevisiae* and yeast²³.

Daily growth rates (DGR) and Specific growth rates (SGR) shrimp fed after 90 day feeding trail with experimental diets clearly demonstrates that DGR were found to be statistically significantly ($P < 0.05$) increased, where as SGR were found to show non significant ($P < 0.01$) increase. The Feed conversion ratio (FCR) values recorded maximum of 2.54 with control diet compared to Probiotic added diet (2.15) and minimum of 1.93 recorded with Biofloc added diets. One way ANOVA results clearly demonstrate that FCR values obtained for experimental diets were statistically significant ($P < 0.05$) compared to control diet. With the addition of either probiotic or Bioflocs, the FCR values were reduced significantly in the present investigation. Similar kind of observation were also reported by several authors in freshwater prawn *M. rosenbergii*, *M. amazonicum* or Penaeid shrimp *L. vannamei*, *P. monodon*, where in which the diets were supplemented with Probiotic bacteria includes *L. bacillus*, *L. acidophilus*, *L. sporogenes*, *Bacillus subtilis*, *B. licheniformis*, *Enterococcus faecium*^{20,24-26}. Feed Efficiency ratio (FER) and Feed conversion Efficiency (FER) values obtained in the present investigation for different types of diets formulated in the present investigation showed a considerable increment in the values for experimental feeds compared to control diet and found to be significant ($P < 0.05$). Similar kind of observations reported by several authors in *M. rosenbergii* after fed with probiotic and Biofloc diets with the supplementation of *Bacillus sps* through diets, in *L. vannamei* with supplementation of *B. licheniformis* and *L. rhamnorus*, molasses as external carbon sources²². Protein Efficiency Ratio (PER) were recorded after the feeding trail experiments are over by 90 days and found to be minimum recorded to be 5.34 with control diet, where as maximum was recorded as 8.18 with Biofloc added trails with *L. vannamei*. One way analysis of variance revealed that variation in PER values between control and Probiotic or Biofloc added groups was found to be statistically significant ($P < 0.05$). So, due to the incorporation of Probiotics i.e. *B. licheniformis* and *L. rhamnorus* and bioflocs using sugarcane molasses as external source of carbon in the present investigation. The results

obtained pertaining to PER gains support from earlier observations with shrimp *M. rosenbergii*, *L. vannamei*, *P. monodon*, fish *Clarius*, *gariepinus*, *Oncorhynchus mykiss*, *Dentex dentex*, *Oreochromis niloticus* using probiotic bacteria including *L. acidophilus*, *L. rhamnorus*, *B. subtilis*, *B. licheniformis*, *Enterococcus faecium*, *B. toyoi* incorporated, yeast *S. cerevisiae* incorporated diets had remarkably enhanced the protein efficiency rate²⁷⁻³⁰. Biofloc addition in the form of Sugarcane molasses also facilitates the growth of Heterotrophic Bacteria in the culture operation, which subsequently acts as supplementary feeding materials, there by significantly increases the protein contents in the candidate sps i.e. the biofloc and Probiotic materials will be converted in to the stuff of the body, there by production rates also significantly ($P < 0.05$) increased. The productivity rates were recorded to be 18.88, 26.64 and 29.79 kgs with Control, Probiotic and Biofloc added fed shrimp groups. One way ANOVA showed that, the variance in productivity among the diets was statistically significant ($P < 0.05$) variation when compared to control group.

The Carbohydrates, Protein and lipids are the main dietary constituents and undergo food oxidation for the production of bulk of energy in the biological system. The energy produced in the biological system, generally be converted into heat and is utilized to maintain the body temperature in a constant way, where as some portion of energy produced will be utilized for the performance of work including muscle contraction, secretory activities, nerve impulse transmission activity, maintenance of internal milieu through regulation of osmotic concentrations and other related metabolic activities. Generally, the food constituents like carbohydrates, lipids play a vital role in providing energy whenever it is being required by the system and the energy was produced into the form of ATP, and energy was stored in high – energy phosphate bonds, and will be used depending on the need and necessity. In general in the living system, including crustaceans, the assimilated energy will be channelled for maintenance of metabolism (R) and production that includes growth assimilation (P), exuvia (E) generation and reproductive activities²⁰. The energy that incurred in metabolic processes, measured through oxygen consumption at organism level and tissue level, is used for the maintenance of both physiological and biochemical function³¹. Growth (P) generally be considered as the energy materially gained by the individual and can be stored as body reserves. The ingested energy may be partitioned into growth, metabolism, excretion, faces formation and will vary among different organisms depending on the factors such as dietary composition and quality of the diet, feeding rate, and food ration^{32,33}. So a balanced energy budget is a tool for bioenergetics modelling in aquaculture and fisheries management³⁴. In the present study, an attempt has been made to study and monitor bio-energetic parameters towards evaluation of energy budget and utilization by shrimp under different feeding trails.

Feeding is an important physiological and biochemical phenomenon associated with the growth of the organism. The rate of feeding is an important for the growth, feed conversion, nutrient retention efficiency and finally contribute towards the proximate composition of the body tissues. It has been observed that determination of the nutrient requirement is affected by feeding rate, and restricted feeding rate will impair not only growth, but also health phenomenon³⁴. The feeding rates were calculated in all the

feeding trial groups and a minimum of 0.581 kcal/g/day recorded with control group, where as a maximum of 0.928 kcal/g/day recorded with probiotic fermented diet with the addition of bioflocs feeding trails group. One way analysis of variance showed that, the variance in feeding rate among the diets selected in the present study was statistically significant ($P < 0.05$) compared to control diet. Soon after the ingested food undergo digestion the digested food materials will be assimilated at the region of gut and remaining fraction are eliminated from the body as faeces. In the present study the absorption rates were recorded as 0.424, 0.602 and 0.654 kcal / g/ day for control, Probiotic fermented and Biofloc added shrimp feeding trails, respectively. One way ANOVA results clearly demonstrates that, the variance in absorption rate among the diets was statistically significant ($P < 0.05$). The feed conversion rate is one of the most important parameter, which plays an important role in the conversion of the feed into the stuff of the body. The feed conversion may be expressed in terms of feed broadcasted for increase in per unit of weight gained by the organism. Soon after the food oxidation is over, the energy produced was categorically expended for maintenance and metabolic processes, the remaining energy was allocated for conversion into the stuff of the body³³. In the present study, the conversion rates were found to be 0.384, 0.474 and 0.548 kcal/g/day, for control, Probiotic fermented and biofloc added diets, respectively. The metabolism is a phenomenon comprises of both anabolism and catabolism, a specific set of chemical reactions responsible for the sustenance of life in biological systems. The metabolic processes allow the organisms to show perfect growth, reproduction, maintenance of life activities and proper response to the surrounding environment. The basic components involved in the metabolic events in the biological system includes availability of molecular oxygen and liberal supply of food constituents for the production of energy through food oxidation. In the present study the metabolic rates were recorded to be 0.313, 0.467 and 0.494 kcal/g/day for Control, Probiotic fermented and Biofloc added feeds. One way ANOVA showed that, the variance in metabolic rates among the diets was statistically significant ($P < 0.05$). Ammonia excretion rate can be considered as a good indicator for the optimum dietary protein content, especially when combined with data on the growth rates for candidate species. Establishing the relationship with protein requirements and ammonia excretion rates, pave way for the reduction of dietary costs and minimize the nitrogenous waste out put³⁴. In the present study the Ammonia excretion rates were found to be 0.041, 0.067 and 0.069 kcal/g/day recorded with control, Probiotic fermented and Biofloc added diets, respectively. The One way ANOVA results clearly demonstrated that, the variance in Ammonia excretion rates among the diets was statistically significant ($P < 0.05$). In the present investigation all the energy utilization performance parameters including Feeding rate, Absorption rate, Conversion rate, Metabolic rate and Ammonia excretion rates were found to be significantly increased ($P < 0.05$) in Probiotic fermented and Biofloc added feeding trails compared to control group. The results obtained gains supports from earlier reports in shrimp *M. rosenbergii*, *L. vannamei*, *P. monodon*, fish *Clarias galiepinus*, *Gncorhynchus mykiss*, *Dentex dentex*, *Oreochromis niloticus*, after fed supplementation with Probiotics including *L. acidophilus*, *L. rhamnorus*, *B. subtilis*, *B. licheniformis*, *Enterococcus faecium*, *B. toyoi*, incorporated yeast *S. cerevisiae*

incorporated diets had remarkably enhanced the energy performance parameters²⁰⁻²⁶.

From the results obtained from the present investigation, it may be concluded that the control diet is formulated with vegetable waste, which includes several dominatory used vegetables obtained from market. Generally, the vegetables are the source of nutrients but are also 75-95% water, consists of biomolecules i.e. lipids, proteins, carbohydrates, minerals, vitamins and Phytonutrients. Vegetables are foods from plants with different uses and are a significant source of nutrients. Generally, the growth performance and production cost of any aquaculture species are depending upon the quality and types of feeds offered respectively. Artificial feeds constitute a major operating cost up to 50% in any culture system. Protein is the largest and most expensive component in formulation of aquaculture diet. Therefore, it is advisable to formulate diets with optimized non-protein sources for basic energy requirement and maximizing protein sparing on growth. The usual strategy in formulating diets for aquaculture is that reducing costs by maximizing the inclusion of carbohydrate up to 50% to sparing expensive protein, which in turn decreases the accumulation of nitrogen waste in culture ponds³⁴. Utilization of dietary carbohydrates from plant sources including soybean meal, rice bran, corn meal, wheat, starch by aquaculture species. Particularly crustaceans are vary³⁵. Due to increased pressure on global fishmeal stocks has generated interest on uses of ingredients, such as vegetables, green leaves, nuts, pulses etc^{36,37}. In the present investigation the vegetable wastes, Probiotics and Bioflocs used for formulation of feeds by replacing fishmeal partially appears to be successful, because the vegetable wastes are catering the needs of growth, moulting, metabolic maintenance where as Probiotics plays an important role in the stimulation of growth potentials, and addition of Bioflocs facilitates the production of heterotrophic Bacterial growth, and planktonic materials will be useful as supplementary feeding materials and subsequently increase the productivity rates. The results obtained gains support from earlier findings includes fishmeal replacement by Soybean meal & complex carbohydrates along with *Bacillus* probiotic strains, incorporation of *Bacillus subtilis*, *Lactococcus lactis*, *Pediococcus pentosecerus* in aquaculture feeds, inclusion of Prebiotics, maize starch, Blue green algae *Arthrospira platensis*, *Chlorella vulgaris*, *Spirulina platensis*, *Azolla pinnata* in aquaculture diets facilitates maximum utilization of feed, there by promotes growth performance, energy utilization in fish and shrimp sps culture operation³⁷. So, the present investigation may be concluded that, use of vegetable wastes for the formulation of diets along with the use of Probiotics and Bioflocs paves way for successful induction of growth potentials and finally contributes for the higher production rates.

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