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## Effects of salinity in a heterotrophic Microbial Floc system on Biofloc nutritional profile and the biochemical composition of *Litopenaeus vannamei*

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### Abstract

Biofloc technology is recognized as sustainable, eco-friendly and economical technology for shrimp aquaculture due to its numerous beneficial outcomes such as disease prevention, maintaining water quality, and promoting growth. The present study was aimed to evaluate the effects of the nutritional profile biofloc and biochemical composition of *Litopenaeus vannamei* under biofloc different salinities. A 45-day investigation was conducted both with and without biofloc at various salinities (10, 20, 30, 35, 40, 50, and 60 ppt). After a few weeks of all treatments and controls, it was discovered that all the essential water quality parameters were within an optimal range. Floc volume was examine during the trial. The nutritional profile biofloc and biochemical composition of *Litopenaeus vannamei* were analyzed at the end of the experiment. Floc volume is increase with day of culture is increases but decrease with higher salinities. Amino and fatty acid profile of biofloc better in lower salinity. The proximate composition of shrimp was found good quality at low salinity. Hence it can be concluded that 10, 20 and 30 ppt under biofloc is better nutritional profile as compared to other salinities. The proximate composition of biofloc treatment shrimp found better than control.

**Keywords:** *L. vannamei*, biofloc, amino acid profile, fatty acid profile, proximate composition, different salinity

### Introduction

Aquaculture is the world's fastest growing food production sector. It contributes about 49.2% of the world's total food fish production and offers over hundreds of millions of people livelihoods, food and nutrition security. The world total fisheries and aquaculture production was reached a recorded 214 million tonnes in 2020. of which, aquaculture production was reported 122.6 million tonnes. World per capita fish consumption reached record of 20.2 kg in 2020. The world's population is rapidly increasing, and by 2050, it is expected to exceed 9 billion, requiring food production to be doubled to meet demand. The global supply of nutritionally balanced and high quality protein food to a growing population is a major challenge. Indian aquaculture is rapidly developing toward the goal of achieving the blue Transformation and ranking second place in the entire globe. India's aquaculture production has increased dramatically, from 0.75 million tonnes in 1950-51 to approximately 12.12 million tonnes of seafood worth Rs 57,586.48 crore today. Shrimp farming is a key player in changing the face of aquaculture around the world. The export of *Litopenaeus vannamei* white leg shrimp has from 5,12,204 MT to 4,92,271 MT in 2020-21. The shrimp aquaculture business is expanding quickly, so it is necessary to develop a nursery rearing system for post larvae (PLs). This can lead to better shrimp seed quality, survival, high disease resistance, decreased environmental stress, and shorter culture times. The biofloc technology is one of the many widely used innovations that shows promise for the growth of sustainable aquaculture. To solve the issue of waste generation and discharge, Biofloc is a sustainable, environmentally friendly, and economically viable technology that can be utilized waste to create a zero water exchange culture system. By adding carbohydrates and maintaining high levels of microbial floc in suspension, this technique helps aerobic decomposition of the organic substance. This boosts the uptake of nitrogen from water and promotes the growth of heterotrophic bacteria, which in turn produces microbial proteins that the shrimp use as food.

A complex community of organic matter combined with other elements to form mass suspended particles is known as biofloc (Cuzon *et al.*, 2004; Emerenciano *et al.*, 2012; Emerenciano *et al.*, 2013b) [4, 8, 10] comprising inorganic compounds (30-40%), such as colloids, organic polymers, bivalent ions, salts, and cell debris, and organic substances (60-70%), a heterogeneous mixture of microbes (fungi, algae, bacteria, protozoa, rotifer, and nematode) (Chu and Lee, 2004) [3]. The variety of the microbes in the biofloc system depends on various factors, including the type of carbon source, the salinity level and the farmed species (Ray *et al.*, 2010) [12]. A number of factors including the environment, carbon sources, floc, bacteria density, and shrimp and fish stocking density, impact the nutritional value of biofloc. Bioflocs are an abundant in terms of vitamins, minerals especially calcium, magnesium and phosphorus. In India, the cultivation of *L.vannamei* is conducted in environments ranging from extremely low salinities range 0-2 ppt to extremely high salinities 50-60 ppt. (CIBA, 2017). Therefore, it's necessary to look over the nutritional profile of biofloc and proximate composition of shrimp under biofloc at different salinity.

## Materials and Methods

To compare the growth and survival of nursery rearing *Litopenaeus vannamei* post larvae under biofloc at different salinities, a 45-day experiment was carried out. The following describes the materials and methodology utilized during the present research work.

- **Experiment laboratory:** The Hands-on Training Centre, Aquaculture, College of Fisheries Science, Veraval, Junagadh Agricultural University, District Gir-Somnath, Gujarat was the location of the experiment. Brine water from the salt pan in Victor Village, Rajula, was used to cultivate the culture. The college lab was used as the location for scientific work.
- **Biofloc production:** Floc inoculums were prepared by following according Avnimelech (1999) [2] technique 20 gm L<sup>-1</sup> pond soil, 10 mg L<sup>-1</sup> ammonium sulphate and 200 mg L<sup>-1</sup> fermented sugarcane molasses (carbon source), within 48 hour inoculums were equally transfer in to the experimental tank at the rate of ratio 1:100 (inoculums: water). Based on the amount and protein content of the feed, the carbon source was determined. Twice a week, this was added. The C:N ratio was maintained more than 15:1 during the experiment. Floc volume was estimated weekly by using of imhoff cone. Healthy Post Larvae of shrimp weighing between 0.021 and 0.029 grams on average were transferred to the biofloc tank at different salinities. A 50-liter square plastic tank was used for the experiment.
- **Experimental design:** Treatment biofloc different salinities was 10 ppt (T1), 20 ppt (T2), 30 ppt (T3), 35 ppt (T4), 40 ppt (T5), 60 ppt (T6), 60 ppt (T7) and control without different salinities 10 ppt (C1), 20 ppt (C2), 30 ppt (C3), 35 ppt (C4), 40 ppt(C5), 60 ppt (C6), 60 ppt (C7) (salinities in ppt) all in triplicate. The experiment was set up using a completely randomized design. Throughout the entire experiment, aeration was given. During the 45-day trial, a zero water exchange system was used, intermittently adding water with the appropriate salinity to maintain the water level in every tank. Throughout the experiment, shrimp PLs were fed commercially available feed containing 35% crude

protein, 5% crude fat, 4% fiber, and 11% moisture at a rate of 5% of their body weight. feeding occurred four times a day at 7:00 PM (morning), 11:00 PM (morning), 15:00 PM (afternoon), and 7:00 AM (evening). At the end of the experiment biofloc was collected and it's used for examined nutritional profile. Shrimp were used for proximate analysis.

- **Nutritional profile of biofloc analysis:** Amino acid profiling-Reagents- a) 100 Mm or 0.1 N HCL (As Diluents): 8.212ml of HCL was taken with 991.788 ml distilled water b) Buffer: 1.115 gm of Tetra-methyl ammonium chloride and 2.035gm of sodium acetate trihydrate were dissolve in 1 L distilled water. The 3.5 pH was adjusted with glacial acetic acid and filtered through 0.45μnylon membrane. c) Organic phase: 1960 ml of acetonitrile was mixed with 40ml methanol d) Mobile phase A: 900 ml of buffer was mixed with 100ml organic phase e) Mobile phase B: 100 ml of buffer was mixed with 900ml organic phasef) 50% NaOH: 5 mg of NaOH pellets dissolved in 100ml distilled water g) Borate buffer: 6.18 gm of boric acid powder was dissolved in 100ml distilled water and The 6.2 pH was adjusted with 50% NaOH h) FMOC Reagent: 100mg of FMOC reagents was diluted in 25 ml of dried acetone. i) n-Hexene

## Sample preparation

**a) Hydrolysis:** Sample biomass (3- 10mg of dry weight or 10-20mg of wet weight) was taken in a heat stable test tube; add 100 μl of 0.1 N HCL, 800 μl of 6N HCL, add 100 μl of norleucine st (1000 ppm) and add 10 μl of phenol and sealed the tube. The hydrolysis was carried out at 110 °C for 60 hour with dry bath. After hydrolysis, the test tube was opened and the contents were transferred to 10m with diluents (0.1N HCL)

**b) Derivation:** After hydrolysis, the 100 μl of hydrolysates or standard or diluent for blank sample were taken into 10ml falcon tube; added 900 μl of borate buffer, 1ml of FMOC and mixed thoroughly; added 4ml of n-Hexene and vortexed for 45 second two layer were formed, upper layer was discarded and lower layer was collected into UHPLC injection tube or vial and seal. Then vials were loaded onto the auto sampler tray ready for analysis.

**c) UHPLC Analysis:** UHPLC vial with collected sample was loaded into the tray of auto sampler. Then 25 μl of sample was injected to an amino acid analyser equipped with column (C 18' 4.6 × 25 mm, 5 μm packing) and dried array detector (265nm Wavelength). The colum was run mobile phase A and B at flow rate of 1.5 ml/ min. The column gradient was maintained as 10-50% B for 45 min., 50% B for 5 min., 90% B for 10 min., 100% B for 2 min., 100% B for 5 min., 10% B for 2 min., 10% B for 6 min. standard amino acid mixture (25 μl) was also run separately and then the chromatograms of standard and sample were compared and quantified.

## Fatty acid profile

### Lipid extraction

Total lipid was extracted by following the Folch (1957) method. The lipids were extracted from muscle. The tissue was homogenized in the 10 volume (of tissue w/v) methanol followed by the 20 volume (of tissue w/v) chloroform in an homogenizer, after dispersion. The whole mixture was

agitated during 15-20 min in an orbital shaker at room temperature.

The homogenate were filtrated (funnel with a folded defatted filter paper) to recover the liquid phase and the filter residue re-homogenized with a second volume of chloroform- methanol. The filtrate were washed with 0.2 volumes (4 ml for 20 ml) of 0.9% NaCl solution and phases are vigorously mixed. The mixture was poured into a separating funnel and allowed to decant. The lower chloroform phase containing lipids were collected and evaporated under vacuum in a rotary evaporator to bring down to a concentration of 2-3 ml. Further evaporation of chloroform was done under a nitrogen stream and residue was weighed to quantify the amount of lipid extracted. The lipid residue was re-dissolved in chloroform/methanol (2:1,v/v) and then stored in a 25 mL conical flask with glass stopper in nitrogen at -20°C until needed.

**Preparation of Fatty Acid Methyl Esters (FAME)** Fatty acid methyl esters (FAME) was prepared from the isolated lipids by heating with the methanolic NaOH first and then with BF3 Methanol for esterification. 5 ml n-heptane was added to recover the methyl esters in organic phase. The mixture was washed with saturated NaCl solution and two phases was separated using a separating funnel. The upper n-heptane phase was pipetted out and stored in 10 ml all glass vials with until further analysis. B) Gas chromatography. The fatty acids profiles were determined in an Agilent Gas Chromatograph, Model 6890N fitted with an Agilent Mass Selective Detector, 5973 series. Separation was carried out in a capillary column (30 x 0.25mm id x 0.25μm DB wax). The starting temperature was 150°C maintained for two minutes at a heating rate of 10OC/minutes. The total running time was 22 minutes. Helium was the carrier gas while the injection volume was 1μL. The fatty acids peaks were identified using Agilent Technologies software 5988-5871EN

### Proximate analysis of shrimp

Proximate composition of shrimp sample was determined by the standard methods (AOAC, 2005). The crude protein was analysed by by Kjeldhal method. Crude lipid was estimated with the by Soxhlet apparatus. Moisture and ash content were determined using the incubtore and muffle furnace respectively.

### Crude protein (CP)

The protein content of the sample was estimated quantitatively by micro Kjeldhal method after acid digestion. The nitrogen content of the sample was estimated constitutively by semi-automatic micro Kjeldhal digestion and distillation apparatus. The crude protein percentage was

obtained by multiplying nitrogen percentage by a factor of 6.25.

$$\text{Crude protein (\%)} = \text{N2 (\%)} \times 6.25$$

Where, N2 is total nitrogen.

**Crude lipid:** The crude lipid was analysed by the ether extract by Soxhlet apparatus using petroleum ether (Boiling point 40-60 °C) as the solvent. The contents of crude lipids were determined gravimetrically after oven drying (80 °C) the extract overnight. The calculation was made as follows:

$$\text{Crude lipid (EE) (\%)} = \frac{\text{Weight of ether extract (g)}}{\text{Weight of the sample (g)}} \times 100$$

**Moisture:** The moisture content of the sample were determined by taking a known weight of sample in Petri-dish and drying in hot air oven at 100-105°C until no change in weight. The moisture content was calculated using following formula:

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample (g)} - \text{Dried weight of sample (g)}}{\text{Wet weight of sample (g)}} \times 100$$

**Ash:** Ash content was estimated by taking a known weight of sample in silica crucible and placing it in a muffle furnace at 600 °C for 6 hours. The calculation was done as follows.

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

### Statistical analysis

Statistical analysis of different growth and physiological parameters were analyzed by one-way analysis of variance (ANOVA) using SPSS VERSION 23.0. Duncan's multiple range tests was used for post hoc comparison of mean ( $P < 0.05$ ) between different groups. All the data presented in the text, figures and tables expressed are mean  $\pm$  standard error and statistical significance of the test was set at  $P < 0.05$ .

### Results

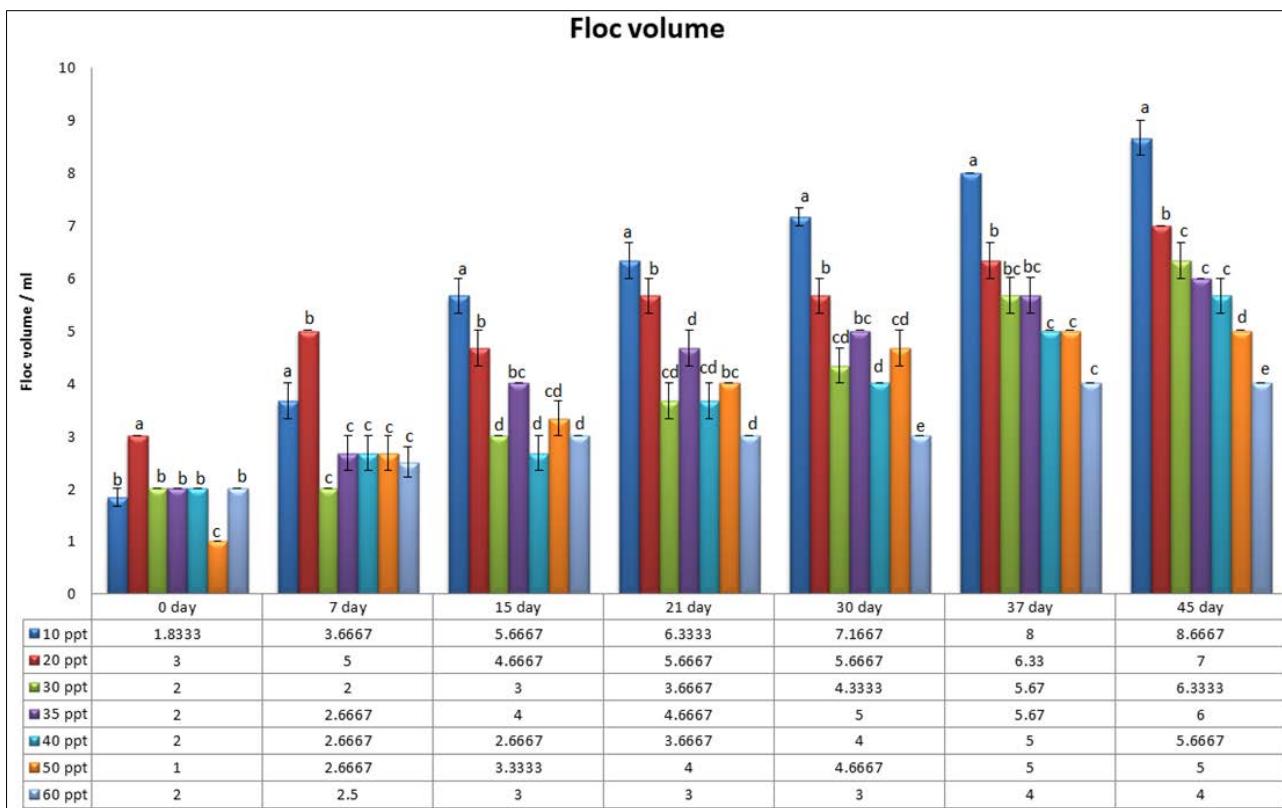
#### Floc volume

Floc volume was estimated weekly by using imhoff cone and observations are presented in table no. 14 and the same is illustrated graphically in fig. 24. The range of the floc volume during the experiment was  $1 \pm 0$  to  $8.66 \pm 0.333$  per ml. The maximum value of floc volume in biofloc treatment 10 ppt  $8.66 \pm 0.333$  and minimum value was 50 ppt  $1 \pm 0$  respectively.

**Table 1:** Floc of water observed during the experiment in treatment biofloc

| Floc volume | 0 day              | 7 day              | 15 day                | 21 day                | 30 day                | 37 day                | 45 day               |
|-------------|--------------------|--------------------|-----------------------|-----------------------|-----------------------|-----------------------|----------------------|
| 10 ppt      | $1.83 \pm 0.166^b$ | $3.66 \pm 0.333^a$ | $5.66 \pm 0.333^a$    | $6.33 \pm 0.333^a$    | $7.16 \pm 0.166^a$    | $8 \pm 0^a$           | $8.6667 \pm 0.333^a$ |
| 20 ppt      | $3 \pm 0^a$        | $5 \pm 0^b$        | $4.66 \pm 0.333^b$    | $5.66 \pm 0.333^b$    | $5.66 \pm 0.333^b$    | $6.33 \pm 0.333^b$    | $7 \pm 0^b$          |
| 30 ppt      | $2 \pm 0^b$        | $2 \pm 0^c$        | $3 \pm 0^d$           | $3.66 \pm 0.333^{cd}$ | $4.33 \pm 0.333^{bc}$ | $5.67 \pm 0.333^{bc}$ | $6.33 \pm 0.333^c$   |
| 35 ppt      | $2 \pm 0^b$        | $2.66 \pm 0.333^c$ | $4 \pm 0^{bc}$        | $4.66 \pm 0.333^d$    | $5 \pm 0^{bc}$        | $5.67 \pm 0.333^{bc}$ | $6 \pm 0^c$          |
| 40 ppt      | $2 \pm 0^b$        | $2.66 \pm 0.333^c$ | $2.66 \pm 0.333^d$    | $3.66 \pm 0.333^d$    | $4 \pm 0^c$           | $5 \pm 0^c$           | $5.66 \pm 0.333^c$   |
| 50 ppt      | $1 \pm 0^c$        | $2.66 \pm 0.333^c$ | $3.33 \pm 0.333^{cd}$ | $4 \pm 0^{bc}$        | $4.66 \pm 0.333^{cd}$ | $5 \pm 0^c$           | $5 \pm 0^d$          |
| 60 ppt      | $2 \pm 0^b$        | $2.5 \pm 0.288^c$  | $3 \pm 0^d$           | $3 \pm 0^d$           | $3 \pm 0^e$           | $4 \pm 0^c$           | $4 \pm 0^e$          |

\*Values are presented as mean  $\pm$  SE

**Fig 1:** Weekly variation in floc volume during the experiment

### Nutritional profile of biofloc analysis

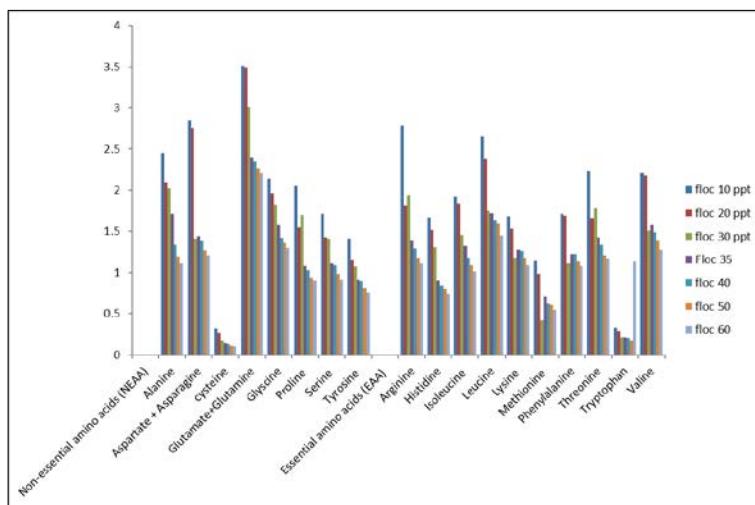
Nutritional profile of biofloc analysis parameters were measured at the end of experiment and presented comprehensively in table no. 2 (amino acid profile) and table no. 3 (Fatty acid profile).

**Amino acid profile:** Amino acid profile mainly two component were analysed non-essential amino acid and essential amino acid. Non-essential amino acid mainly alanine, aspartate, asparagine, cysteine, glutamate,

glutamine, glycine, proline, serine and tyrosine maximum value was 2.45, 2.15, 0.17, 3.15, 2.14, 2.05, 1.17, 1.41 and minimum 1.11, 1.21, 0.1, 2.21, 1.3, 0.9, 0.91, 0.76 respectively. Essential amino acid mainly arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine maximum value was 2.78, 1.67, 1.52, 2.65, 1.91, 0.64, 1.71, 2.23, 0.33, 2.21 and minimum 1.11, 0.74, 1.01, 1.45, 1.09, 0.55, 1.08, 1.17, 1.13, 1.28 respectively.

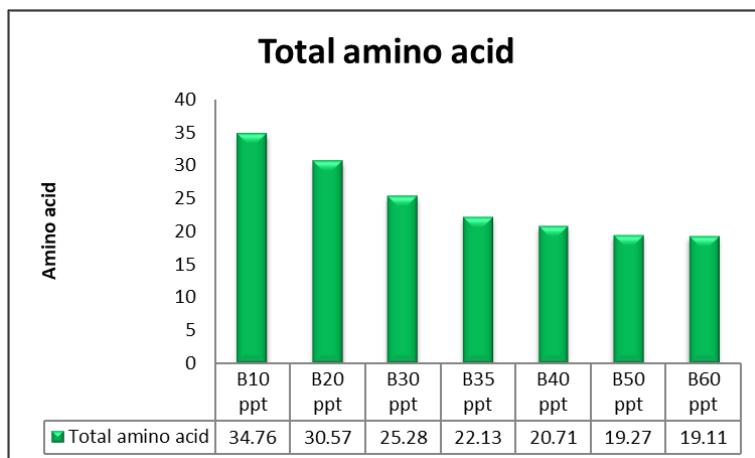
**Table 2:** Amino acid profiling of biofloc

| Amino acid                              | floc 10 ppt | floc 20 ppt | floc 30 ppt | Floc 35 | floc 40 | floc 50 | Floc 60 |
|---|-------------|-------------|-------------|---------|---------|---------|---------|
| <b>Non-essential amino acids (NEAA)</b> |             |             |             |         |         |         |         |
| Alanine                                 | 2.45        | 2.10        | 2.02        | 1.71    | 1.34    | 1.19    | 1.11    |
| Aspartate + Asparagine                  | 2.85        | 2.75        | 1.41        | 1.44    | 1.38    | 1.27    | 1.21    |
| Cysteine                                | 0.32        | 0.27        | 0.17        | 0.14    | 0.13    | 0.11    | 0.1     |
| Glutamate+Glutamine                     | 3.51        | 3.49        | 3.01        | 2.4     | 2.35    | 2.26    | 2.21    |
| Glycine                                 | 2.14        | 1.96        | 1.82        | 1.58    | 1.42    | 1.36    | 1.3     |
| Proline                                 | 2.05        | 1.55        | 1.7         | 1.08    | 1.03    | 0.94    | 0.9     |
| Serine                                  | 1.71        | 1.43        | 1.41        | 1.11    | 1.09    | 0.98    | 0.91    |
| Tyrosine                                | 1.41        | 1.15        | 1.07        | 0.91    | 0.89    | 0.81    | 0.76    |
| <b>Essential amino acids (EAA)</b>      |             |             |             |         |         |         |         |
| Arginine                                | 2.78        | 1.81        | 1.94        | 1.39    | 1.29    | 1.18    | 1.11    |
| Histidine                               | 1.67        | 1.52        | 1.31        | 0.9     | 0.84    | 0.8     | 0.74    |
| Isoleucine                              | 1.92        | 1.83        | 1.46        | 1.32    | 1.18    | 1.09    | 1.01    |
| Leucine                                 | 2.65        | 2.38        | 1.75        | 1.72    | 1.64    | 1.59    | 1.45    |
| Lysine                                  | 1.68        | 1.53        | 1.18        | 1.28    | 1.26    | 1.18    | 1.09    |
| Methionine                              | 1.14        | 0.98        | 0.42        | 0.71    | 0.62    | 0.61    | 0.55    |
| Phenylalanine                           | 1.71        | 1.69        | 1.11        | 1.22    | 1.22    | 1.13    | 1.08    |
| Threonine                               | 2.23        | 1.66        | 1.78        | 1.43    | 1.34    | 1.21    | 1.17    |
| Tryptophan                              | 0.33        | 0.29        | 0.21        | 0.21    | 0.2     | 0.17    | 0.13    |
| Valine                                  | 2.21        | 2.18        | 1.51        | 1.58    | 1.49    | 1.39    | 1.28    |
| Total                                   | 34.76       | 30.57       | 25.28       | 22.13   | 20.71   | 19.27   | 19.11   |

**Fig 2:** Amino acid profiling of biofloc

The total amino acid value 10 ppt, 20 ppt, 30 ppt, 35 ppt, 40 ppt, 50 ppt and 60 ppt were 33.24, 29.67, 25.28, 22.13,

20.71, 19.27 and 19.11 respectively. The total amino acid was decrease with higher level of salinity.

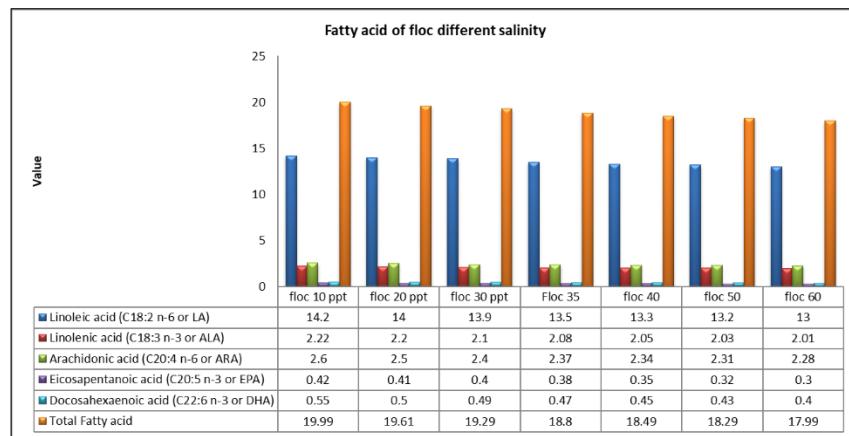
**Fig 3:** Total amino acid profiling of biofloc

**Fatty acid profile:** Fatty acid profile of biofloc was analysed at different salinity. Mainly five component was analysed linoleic acid (LA), linolenic acid (ALA), arachidonic acid (ARA), eicosapentanoic acid (EPA), docosahexaenoic acid (DHA) maximum value was 14.2,

2.22, 2.6, 0.42, 0.55 and minimum 13, 2.02, 2.28, 0.3, 0.4 respectively. The total fatty acid value 10 ppt, 20 ppt, 30 ppt, 35 ppt, 40 ppt, 50 ppt and 60 ppt were 19.99, 19.61, 19.29, 18.8, 18.49, 18.29 and 17.99 respectively.

**Table 3:** Fatty acid profiling of biofloc

| Essential FA                            | floc 10 ppt | floc 20 ppt | floc 30 ppt | Floc 35 ppt | floc 40 ppt | floc 50 ppt | floc 60 ppt |
|---|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Linoleic acid (C18:2 n-6 or LA)         | 14.2        | 14          | 13.9        | 13.5        | 13.3        | 13.2        | 13          |
| Linolenic acid (C18:3 n-3 or ALA)       | 2.22        | 2.2         | 2.1         | 2.08        | 2.05        | 2.03        | 2.01        |
| Arachidonic acid (C20:4 n-6 or ARA)     | 2.6         | 2.5         | 2.4         | 2.37        | 2.34        | 2.31        | 2.28        |
| Eicosapentanoic acid (C20:5 n-3 or EPA) | 0.42        | 0.41        | 0.4         | 0.38        | 0.35        | 0.32        | 0.3         |
| Docosahexaenoic acid (C22:6 n-3 or DHA) | 0.55        | 0.5         | 0.49        | 0.47        | 0.45        | 0.43        | 0.4         |
| Total Fatty acid                        | 19.99       | 19.61       | 19.29       | 18.8        | 18.49       | 18.29       | 17.99       |

**Fig 4:** The graph of fatty acid profiling of biofloc at the end of experiment.

### Proximate analysis

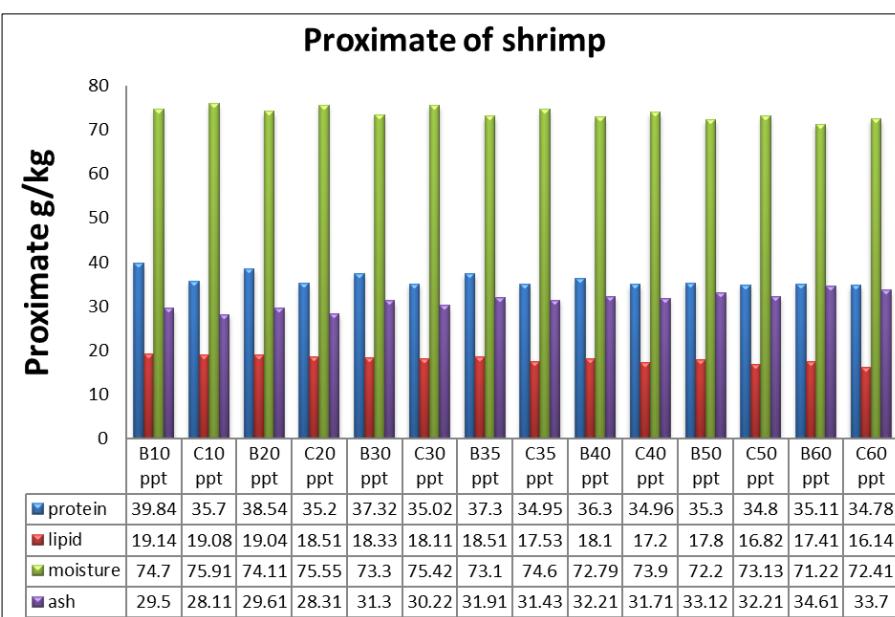
#### Proximate composition of shrimp

Proximate composition of shrimp analysis parameters was measured at the end of experiment and presented comprehensively in table no. 4 and fig no.5 Proximate composition of shrimp four component were analysed mainly Proximate composition biofloc treatment of shrimp mainly protein, lipid, moisture and ash maximum value was 39.84, 19.14, 74.70 and 29.50 and minimum 35.11, 17.41, 71.22, 34.61 respectively. Proximate composition control shrimp mainly protein, lipid, moisture and ash maximum value was 35.7, 19.08, 75.91 and 28.11 minimum 34.78, 16.14, 72.41 and 33.70 respectively.

### Proximate analysis

**Table 4:** Proximate composition shrimp parameters observed after the experiment in treatment and control

| Biofloc  | B10 ppt | B20 ppt | B30 ppt | B35 ppt | B40 ppt | B50 ppt | B60 ppt |
|----------|---------|---------|---------|---------|---------|---------|---------|
| Protein  | 39.84   | 38.54   | 37.32   | 37.30   | 36.30   | 35.30   | 35.11   |
| Lipid    | 19.14   | 19.04   | 18.33   | 18.51   | 18.10   | 17.80   | 17.41   |
| Moisture | 74.70   | 74.11   | 73.303  | 73.10   | 72.79   | 72.20   | 71.22   |
| Ash      | 29.50   | 29.61   | 31.303  | 31.91   | 32.21   | 33.12   | 34.61   |
| Control  | C10 ppt | C20 ppt | C30 ppt | C35 ppt | C40 ppt | C50 ppt | C60 ppt |
| Protein  | 35.7    | 35.20   | 35.02   | 34.95   | 34.96   | 34.80   | 34.78   |
| Lipid    | 19.08   | 18.51   | 18.11   | 17.53   | 17.20   | 16.82   | 16.14   |
| Moisture | 75.91   | 75.55   | 75.42   | 74.60   | 73.90   | 73.13   | 72.41   |
| Ash      | 28.11   | 28.31   | 30.22   | 31.43   | 33.71   | 32.21   | 33.70   |

**Fig 5:** The proximate composition of shrimp at the end of experiment

### Discussion

At the start of the experimental period, biofloc volume was minimal, but it gradually increased as the culture progressed due to the accumulation of organic matter and the growth of microbial populations. In contrast, an inverse relationship was observed between salinity and floc volume, with higher salinity levels causing a reduction in floc formation. Overall, floc development showed a positive association with the duration of the culture period. Evaluation of the biofloc

nutrient composition confirmed the presence of a wide range of amino acids, including both non-essential and essential forms. The predominant non-essential amino acids comprised alanine, aspartate, asparagine, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine. Essential amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine were also identified. The concentration of these amino acids was influenced by

salinity and exhibited a consistent decline at higher salinity levels. A comparable reduction was noted in fatty acid profiles, including a decrease in total fatty acid content with increasing salinity. Shrimp cultured in the biofloc system demonstrated enhanced nutritional characteristics, with increased protein, lipid, and ash contents compared to shrimp maintained under control conditions. In contrast, moisture content was higher in the control group. These observations indicate that the biofloc system contributed to improved shrimp body composition. Additionally, increasing salinity resulted in reduced protein, lipid, and moisture levels in shrimp, while ash content showed an upward trend. These patterns align with previously reported findings by Xu and Pan (2012) [13].

### Conclusion

Overall, the biofloc culture system exhibited improved floc accumulation over time, although higher salinity levels negatively affected floc volume. Nutritional assessments revealed that non-essential amino acids, essential amino acids, total amino acids, essential fatty acids, and total fatty acids progressively declined as salinity increased. Among all treatments, the most favorable nutritional composition was recorded at 10 ppt salinity. Shrimp proximate composition was also influenced by salinity, with optimal protein, lipid, and moisture levels observed at 10 ppt, whereas maximum ash content was recorded at 60 ppt salinity.

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