NUTRITION AND FEEDING STRATEGY IN A BIOFLOC SYSTEM

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Biofloc Technology (BFT) is an environmental friendly aquaculture system since in this farming method nutrients could be continuously recycled and reused. This system is dominated by heterotrophic (HS) microorganisms compared to traditional autotrophic (AS) microalgae. BFT is beneficial by the use of minimum or zero water exchange. The HS has two major roles: (i) maintenance of water quality, by the uptake of nitrogen compounds generating "*in situ*" microbial protein; and (ii) nutrition, increasing culture feasibility by reducing feed conversion ratio and a decrease of feed costs. The water quality of biofloc system is maintained by control of bacterial community over autotrophic microorganisms which is achieved using a high carbon to nitrogen ratio (C:N). Nitrogenous by-products can be easily taken up by heterotrophic bacteria in the presence of adequate quantities of carbon. High carbon to nitrogen ratio (~15-20:1) is thus essential for optimum heterotrophic bacterial growth, using this energy for maintenance (respiration, feeding, movement, digestion, etc) and also for growth and for multiplication to produce new bacterial cells. High carbon quantities in water could supersede the carbon assimilatory capacity of algae, there by contributing to bacteria growth. Aerobic microorganisms are more efficient in converting feed to new cellular mass (40-60% of conversion efficiency), rather than higher organisms which spend about 10-15% for increase in weight. Bacteria and other microorganisms act as efficient "biochemical systems" to degrade and metabolize organic residues. In other words, they recycle very efficiently nutrients in a form of organic and inorganic matter (un-consumed and non-digested feed, metabolic residues and carbon sources applied as fertilizers) into new microbial cells.

The particulate quantities of organic matter and other organisms in microbial food web are potential food sources for aquatic animals like shrimp. The macro aggregates (biofloc) is a rich protein-lipid natural source available "*in situ"* throughout the day. The BFT is formed in the water column due to the complex interaction between organic nutrients, physical substrate and variety of microorganisms such as plankton, free and attached bacteria, conglomerates of particulate organic matter and also grazers, such as rotifers, flagellates and ciliates and protozoa and copepods. This natural productivity plays an important role for recycling nutrients and maintaining the water quality and providing nutrition to the candidate aquatic species like shrimp.

Biofloc as Nutrient

The consumption of biofloc by shrimp has demonstrated several benefits such as enhancement of growth rate, reduction of FCR and thereby decreased feed costs. Growth improvement has been attributed to nutritional components of both bacterial and algae. This biofloc nutrition will reduce up to 30% of conventional feeding ration consumption in shrimp. The short-term feeding study using ^{15}N-nitrogen enrichment has demonstrated that bioflocs could contribute substantially to the nutrition of *L. vannamei.* Moreover, the consumption of bioflocs can increase feed utilization efficiency by recovery of some fraction of excreted nutrients and nitrogen retention from added feed by 7–13%. Biofloc technology also helps in rationalizing the feeding rates. The results of feeding rates of (10%, 9.5%, 9.0%, 8.5%, and 8.0% body weight day^{-1} in biofloc system) were compared with control at 10% feeding level but without biofloc indicated that 8% feeding rate performed on par with control.

In tilapia feed utilization is higher in BFT at a rate of 20% than conventional water-exchange systems. These results of BFT have driven opportunities to use alternative diets. The feeds were formulated with low nutritional densities especially for intensive biofloc systems. This has increased the scope to reduce protein levels in the feeds and also to use alterative feed ingredients by replacing different marine protein sources (i.e. fishmeal, squid meal, etc) leading "green" market opportunities.

In biofloc particles, protein, lipid and ash contents vary substantially (12 to 49, 0.5 to 12.5 and 13 to 46%, respectively. The same trend of wide variation also occurs with fatty acids (FA) profile. Essential FA such as linoleic acid (C18:2 n-6 or LA), linolenic acid (C18:3 n-3 or ALA), arachidonic acid (C20:4 n-6 or ARA), eicosapentanoic acid (C20:5 n-3 or EPA) and docosahexaenoic acid (C22:6 n-3 or DHA), as well as sum of n-3 and sum of n-6 differ considerably between 1.5 to 28.2, 0.04 to 3.3, 0.06 to 3.55, 0.05 to 0.5, 0.05 to 0.77, 0.4 to 4.4 and 2.0 to 27.0% of total FA. Type of carbon source, freshwater or marine water and production of biofloc biomass (in bioreactors or culture tanks) definitely influence the FA profile. Information is still scarce about how microorganisms profile and its nutritional contents could impact shrimp growth.

The amino acid composition of the biofloc produced from different carbohydrate sources like maida, wheat, gram flour, ragi, rice flour, corn flour, molasses and multigrain atta. The result revealed higher level of essential amino acids in ragi, molasses and multigrain atta compared to other treatments. The utilization of protein by the shrimp depends on the availability of essential amino acids in the diet. Among them certain amino acids like arginine, methionine and lysine are limiting amino acids as they are generally deficient in the diet. The biofloc samples from ragi, molasses and multigrain atta are having higher arginine, methionine and lysine levels of 1.35, 1.12, 2.13; 1.32, 1.17, 1.66 and 1.25, 1.093, 1.663 per 100 g dry floc, respectively compared to other treatments and control. Because of higher essential amino acids, the essential amino acid index is also better in biofloc reared with these carbohydrate sources viz., ragi, molasses and multigrain atta.

Table: Amino acid composition (g/100g dry floc) of the biofloc produced with different carbon sources

	C1-Maida	C2-Wheat	C3-Gram Flour	C4-Ragi	C5-Rice flour	C6-Corn flour	C7-Molasses	C8-Multigrain Atta
Essential Amino acids								
Arg	1.143	1.187	1.113	1.353	1.160	1.273	1.320	1.253
His	2.803	2.663	2.740	2.920	2.567	2.440	3.030	2.907
Ile	1.313	1.307	1.193	1.317	1.240	1.173	1.360	1.293
Leu	2.150	2.057	2.050	2.137	1.493	1.727	2.153	1.900
Lys	1.393	1.343	1.463	1.560	1.270	1.257	1.660	1.663
Met	1.007	0.967	1.077	1.127	0.903	0.863	1.170	1.093
Phe	1.547	1.860	1.717	1.803	1.647	1.507	1.773	1.790
Thr	1.653	1.750	1.730	1.870	1.850	1.750	1.810	1.850
Trp	0.290	0.290	0.290	0.330	0.280	0.323	0.367	0.350
Val	1.383	1.373	1.310	1.490	1.373	1.277	1.367	1.313
Non essential amino acids								
Ala	2.677	2.560	2.227	2.267	2.450	2.430	2.287	2.277
Asp	2.863	2.493	2.433	2.553	2.673	2.327	2.367	2.373
Cys	0.417	0.393	0.437	0.453	0.370	0.517	0.457	0.477
Glu	3.277	3.617	3.663	3.407	3.060	3.367	3.433	3.600
Gly	2.183	2.443	2.303	2.150	2.820	3.383	3.463	3.427
Pro	1.290	1.293	1.270	1.287	1.367	1.437	1.290	1.313
Ser	1.350	1.550	1.253	1.383	1.483	1.383	1.440	1.360
Tyr	1.223	1.053	1.133	0.963	1.000	0.987	1.147	1.163

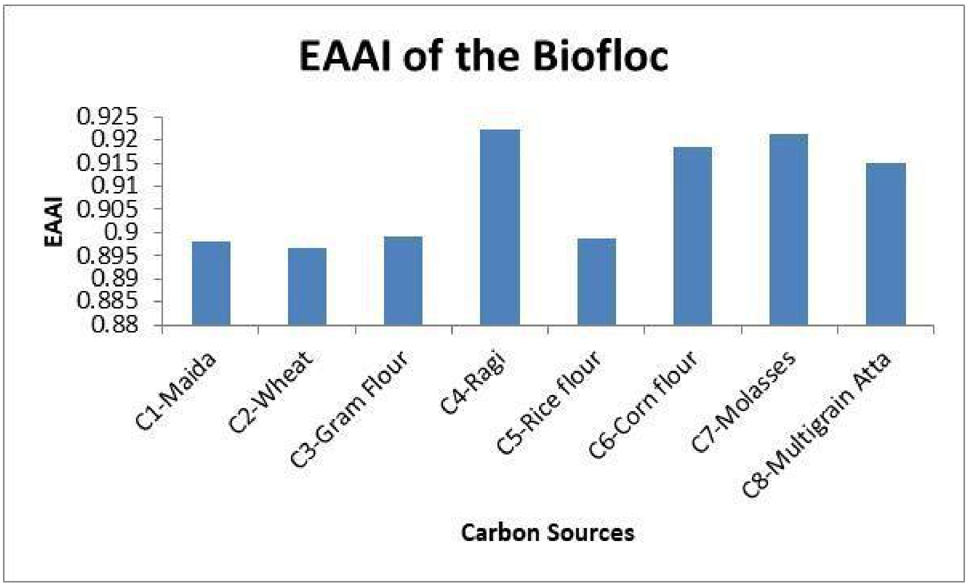

Fig. 1. Essential Amino Acid Index (EAAI) of the biofloc produced from different carbon sources

Role of Biofloc on dietary protein levels in shrimp feeds

Dietary protein levels in the diets of whiteleg shrimp reared in clear seawater system has been studied extensively. Typically commercial shrimp feed contain 38–42% of CP in Asian countries. On the other hand, as far as we know, only few studies have investigated the optimum dietary protein level for whiteleg shrimp reared in bioflocs system. These studies demonstrated the positive contribution of bioflocs aggregates on the optimum dietary protein level in whiteleg shrimp. It was suggested to substitute high protein (40%) with low-protein (30%) feed in the nursery phase. The other study reported dietary protein level could be reduced to 25% without affecting the growth of juvenile whiteleg shrimp reared in bioflocs system. The level of total suspended solids (TSS) also influences the protein level in the diet. Recommended level of TSS was below 500 mg L^{-1} for shrimp culture.

The bioflocs tanks fed with high dietary CP (40%) than that of low CP (30%) had higher in nitrite, nitrate and phosphate content and TAN content showed increasing pattern with the corresponding increase in dietary protein level, since high-protein feed would have generated more TAN than the low-protein feed ammonia oxidizing bacteria developed faster than nitrite oxidizing bacteria. In the BFT, the formation of the bioflocs was likely to be linked with the direct assimilation of dissolved nitrogenous matters (especially ammonia–nitrogen) from diets and shrimp excretions by heterotrophic bacteria.

It was showed that juveniles of *L. vannamei* fed with 35% CP feed grew significantly better in biofloc conditions as compared to clear-water conditions. The study performed in a heterotrophic-based condition detected no significant difference in FCR when feeding *L. vannamei* 30% and 45% CP diets. These results indicate, floc biomass might provide a complete source of cellular nutrition as well as various bioactive compounds even at high density. Growth might be enhanced by continuous consumption of "native protein", protein source without previous treatment, which could possess a "growth factor" similar to the one investigated in squid. Is well known that protein, peptides and amino acids participate fully in synthesis of new membranes, somatic growth and immune function and biofloc can potentially provide all such nutrients.

However, is already known that microorganisms in biofloc might partially replace protein content in shrimp diets, although were not always the case. Recent studies determined how lowering the protein content of diet would affect growth performance of shrimp reared in biofloc conditions. In one of the study it was found that at least 10% of protein content in feed can be reduced when *F. paulensis* postlarvae are cultured in BFT conditions. It was observed that shrimp fed with less than 25% crude protein under biofloc conditions performed similarly to shrimp raised under regular clear-water intensive culture with a 37%-protein diet. The biofloc system also delivered better consistent survival rates, especially at higher density. A low-protein biofloc meal-based pellet (25% CP) was evaluated as a replacement of conventional high-protein fishmeal diet (40% CP) for *L. vannamei* under biofloc conditions. The results showed that is possible to replace 1/3 part of a conventional diet by alternative low-protein biofloc meal pellet without interfering survival and shrimp performance.

Our experimental results with graded levels of protein in a biofloc system have indicated that the dietary protein level could be reduced to 32% without reduction of performance compared to 40% dietary protein level.

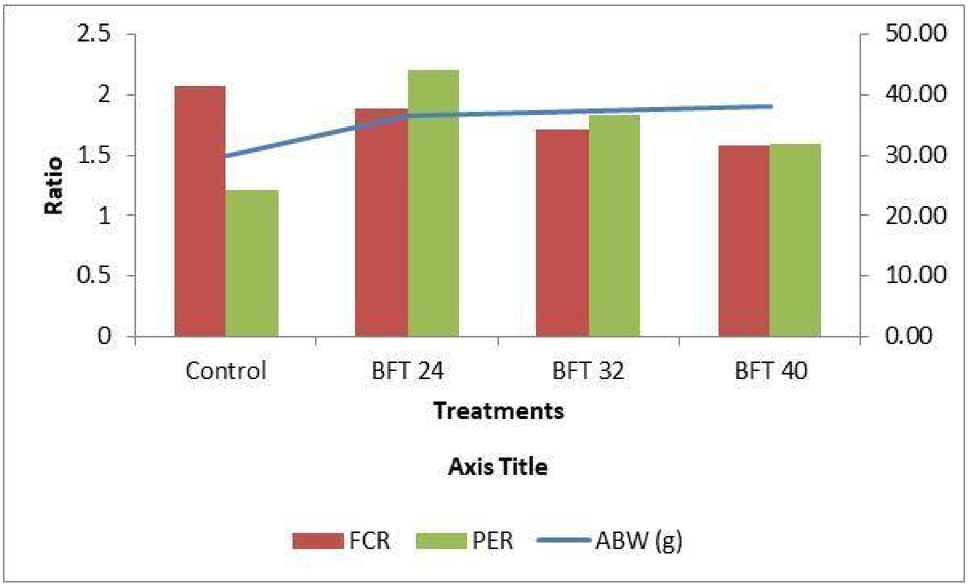

Fig. 2. Role of Biofloc in replacing fishmeal in the feed

The major limiting factor for aquaculture growth will be the availability of fishmeal and fish oil for aquafeed production in the years to come. Hence the fish nutritionists are trying to find alternatives to the Fishmeal and fish oil. This inspired researchers to develop the biofloc technology, which is also applicable to intensive and semi-intensive systems. With biofloc technology, where nitrogenous waste generated by the cultivated organisms is converted into bacterial biomass (containing protein), *in situ* feed production is stimulated through the addition of an external carbon source. Although bioflocs show an adequate protein, lipid, carbohydrate and ash content for use as an aquaculture feed, more research is needed on their amino acid and fatty acid composition. Now, fishmeal and fish oil supply essential amino acids (such as lysine and methionine) that are deficient in plant proteins and fatty acids (eicosapentanoic acid and docosahexanoic acid) not found in vegetable oils. Although bioflocs meet nutritional standards to serve as a aquaculture feed in general, research has shown that the capacity of the technique to control the water quality in the culture system and the nutritional properties of the flocs are influenced by the type of carbon source used to produce the flocs. Different organic carbon sources each stimulated specific bacteria, protozoa and algae, and hence influenced the microbial composition and community organization of the bioflocs and thereby also their nutritional properties. Feeding experiments revealed that besides these characteristics, the type of carbon source also influenced the availability, palatability and digestibility for the cultured organisms. However, further research should focus on the use of low-cost non-conventional agro-industrial residues as carbon source and hence upgrade waste to nutritious feed. Different carbon sources will stimulate the growth of the indigenous microbiota in another way and thus exert a distinctive effect on water quality, *in situ* feed production and utilization of the flocs by the cultured organisms. In addition, not only the carbon source, but also the indigenous microbiota present in the pond water will put forth a characteristic effect that needs to be considered. An important factor here is to determine the role of algae and their interaction with the bacteria in the bioflocs.

An interesting topic for further research could be the identification of micro-organisms (bacteria and micro-algae) that are able to produce bioflocs with the desired nutritional properties and a good ability to control the water quality. Such micro-organisms could be used as an inoculum for the start-up of aquaculture systems with biofloc technology. All these findings and possible modus operandi emphasize the need for further study of biofloc composition in order to achieve a desired nutritional outcome, since different research groups have obtained different results in respect to biofloc nutritional composition.

The BFT also helped in replacing fish meal up to 40% in shrimp diets. In addition efforts were also made to use biofloc meal as the feed ingredient in shrimp feeds by replacing fish meal. In a trial performed in clear-water conditions detected that fishmeal can be completely replace with soy protein concentrate and biofloc meal (obtained from super-intensive shrimp farm effluent) in 38% CP diets without adverse effects on *L. vannamei* performance. Moreover, observed that biofloc produced in bioreactors using tilapia effluent and sugar as a growth media could offer an alternative protein source to shrimp feeds. Microbial floc-based diets significantly outperformed control fishmeal-based diets in terms of weight gain per week with no differences in survival.

Role of Biofoc on Broodstock Nutrition

The BFT has been successfully applied for grow-out, but the information little is known about biofloc benefits on breeding. The nutritional problems of brood stock and biosecurity issues were remain unresolved and alternatives should be evaluated for broodstock. Breeders raised in BFT limited or zero water exchange system are nutritionally benefited by the natural productivity (biofloc) available continuous *in situ* nutrition during the whole life-cycle. Biofloc in a form of rich-lipid-protein source could be utilized for initial stages of broodstock's gonads formation and ovary development. The continuous availability of nutrients especially fatty acids which are not oxidized could promote high nutrient storage in hepatopancreas, transferred to hemolymph and directed to ovary, resulting in a better sexual tissue formation and reproduction activity. Furthermore, production of broodstock in BFT could be located in small areas close to hatchery facilities, preventing spread of diseases caused by shrimp transportation. In conventional systems breeders used to be raised in large ponds at low density. However, risks associated with accumulation of organic matter, cyanobacteria blooms and fluctuations of some water quality parameters (such as temperature, DO, pH and N-compounds) remains high and could affect the shrimp health in outdoor facilities. Once the system is stable BFT provides stabilized parameters of water quality when performed in indoor facilities such as greenhouses, guaranteeing shrimp health.