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The Effect of Microbial Biofloc on Water Quality, Survival and Growth of the Green Tiger Shrimp (*Penaeus Semisulcatus*) Fed with Different crude Protein Levels

I: Sustainable Solution to the Dependency on Fish Oil, Fishmeal and Environmental Problems

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ABSTRACT

On- farm trial were conducted to evaluate the effects of feeding on pellets with different protein levels in the presence and absence of the bioflocs on water quality, survival and growth of the green tiger shrimp (*Penaeus semisulcatus*) in intensive types of shrimp culture systems. Five different feeds were formulated. Four biofloc treatments (BFT) and one control were managed in 10 greenhouses earthen ponds of 300 m^2 each: BFT fed feeds of 31.15% crude protein (CP) ($BFA_{31.15\%}$), 21.60% CP ($BFB_{21.60\%}$), 18.45% CP ($BFC_{18.45\%}$) and 16.25% CP ($BFD_{16.25\%}$) and a control without biofloc but fed a 42.95% CP feed. The bioflocs were developed in the BFT treatments using wheat flour as a carbon source. Thirty juvenile *Penaeus semisulcatus* with an average body weight of 1.55 ± 0.2 g were stocked per m^2 and each dietary treatment and the control was tested in two replicates over a 120 days feeding trial. Biofloc diets enhanced shrimp growth. There was a significant differences (P<0.05) between control and treatment groups in terms of final average body weight (ABW) at harvest (15.1 ± 1.6; 19.8 ± 0.1; 19.2 ± 0.3; 20.1 ± 0.3 and 19.0 ± 1.1 for control; $BFA_{31.15\%;}$ $BFB_{21.60\%;}$ $BFC_{18.45\%}$ and $BFD_{16.25\%}$, respectively) confirming the utilization of biofloc by shrimp as a food source. Concerning final shrimp yield per dietary treatment, there was a significant differences (P<0.05) between control and treatment groups at harvest (114.1 ± 5.3; 151.5 ± 7.9; 152.6 ± 7.4; 155.5 ± 6.9 and 153.6 ± 8.4 kg $pond^{-1}$ for control; $BFA_{31.15\%;}$ $BFB_{21.60\%;}$ $BFC_{18.45\%}$ and $BFD_{16.25\%}$, respectively). The FCR differs significantly between BFT treatment and control (P<0.05). Growth (weight gain $week^{-1}$) for control, $BFA_{31.15\%,}$ $BFB_{21.60\%,}$ $BFC_{18.45\%}$ and $BFD_{16.25\%}$ were respectively 0.85 ± 0.4; 0.98 ± 0.1; 1.00 ± 0.2; 1.11± 0.2; and 1.00 ± 0.2. The shrimp survival % was not affected *(P*>0.05) by the treatments and it ranged between 85.7% and 88.4%. The addition of carbohydrate to the water column (P<0.05) reduced the nitrate, nitrite-N levels and TAN in the experimental greenhouses. There was a significant differences (P<0.05) in the nitrate, nitrite-N levels and TAN concentrations between treatments and the control greenhouses. Simple economic calculation revealed that the lowest CP% BFT treatment, $BFD_{16.25\%}$ had a lowest total feed cost in addition to better water quality and best shrimp economical production compared to conventional control diet. The results concluded also that the biofloc treatments succeeded to reduce the cost of kg shrimp production. Tukey's HSD test revealed also that the four microbial floc diets significantly ($P<0.05$) outperformed control in terms of final ABW, final yield (kg shrimp/pond), FCR, weight gain per week and water quality parameters with no differences in survival rate.

Keywords: Biofloc, sustainability, Alternative feeds, water quality, Green tiger shrimp

INTRODUCTION

World Aquaculture is growing with an annual rate of 8.9–9.1% since the 1970s. This high growth rate is needed to solve the problem of shortage in protein food supplies, which is particularly situated in the developing countries (Gutierrez-Wing and Malone, 2006; Matos *et al.*, 2006 and Subasinghe, 2005). The global shrimp market has expanded from less than $1 billion to $5.8 billion (US) from 2000 to 2005 (FAO, 2008). To meet this growing demand, the shrimp industry is shifting from extensive rearing systems to more intensive rearing systems. However, environmental (i.e. discharge of farm effluents) and economical limitations (Higher prices of feed ingredients, especially fishmeal) can hamper this growth. The expansion of the aquaculture production is restricted due to the pressure it causes on the environment by the discharge of waste products in the water bodies and by its dependence on fish oil and fishmeal (De Schryver *et al*, 2008).

In order for aquaculture to be completely successful, the industry will need to develop technology that will increase economic and environmental sustainability (Kuhn *et al.*, 2010). This technology implements cheaper alternative ingredient to fishmeal and this will effectively reduce the costs of feed as feed costs can account for 50% of operational expense (Van Wyk *et al.*, 1999) while reducing their impact on overexploited natural fisheries (Tacon *et al.*, 2006 and Naylor *et al.*, 2009). Thus, it is important to determine if alternative ingredients derived from biologically treating fish waste, bioflocs (microbial flocs), could be a suitable replacement ingredient in marine shrimp diets. If implemented successfully, this option would offer a sustainable option to fishmeal.

According to Kuhn *et al.* (2010), initial cost estimates for biofloc production is approximately $400 to $1000 per ton of dry ingredients which is projected to be less than the ingredients such as fishmeal and within the range for soybean meal. Over the period of January 2008 to May 2009, the global fishmeal market varied from a low mean of about $900 to a high mean of $1250 per metric ton. During the same time frame, soybean meal varied approximately from a low mean of $375 to a high mean of $550 (FAO, 2009). Thus, we are suggesting that the use of biofloc represents a viable and more sustainable feed option due to cost, the manner in which it is generated, and the potential that it can ease the pressure on wild fisheries by reducing at least some of the demand for fishmeal.

Aquaculture produces large quantities of wastes that contain solids (e.g. feces and uneaten feed) and nutrients (e.g. nitrogen and phosphorus) which can be detrimental to the environment, if managed improperly. These solids and nutrients originate from uneaten feed, feces, and animal urea/ammonia (Maillard *et al*., 2005 and Sharrer *et al*., 2007), if released directly to the environment, these solids and nutrients can be pollutants resulting in environmental issues such as eutrophication (Wetzel, 2001) or could be directly toxic to aquatic fauna (Timmons *et al*., 2002 and Boardman *et al*., 2004). The most common method for dealing with this pollution has been the use of continuous replacement of the pond water with new clean water from the water source (Gutierrez-Wing and Malone, 2006). For instance, Penaeid shrimp require about 20 m^3 fresh water per kg shrimp produced (Wang, 2003). For an average farm with a production of 1000 kg shrimp ha^{-1} yr^{-1} and total pond surface of 5 ha, this corresponds with a water use of ca. 270 m^3 day^{-1}.

A relatively new alternative to previous approaches is the bioflocs technology (BFT) aquaculture (Avnimelech, 2006). In these systems, a co-culture of heterotrophic bacteria and algae is grown in flocs under controlled conditions within the culture pond. The system is based on the knowledge of conventional domestic wastewater treatment systems and is applied in aquaculture environments. Microbial biomass is grown on fish excreta resulting in a removal of these unwanted components from the water. The major driving force is the intensive growth of heterotrophic bacteria. They consume organic carbon; 1.0 g of carbohydrate-C yields about 0.4 g of bacterial cell dry weight-C; and depending on the bacterial C/N-ratio thereby immobilize mineral nitrogen. As such, Avnimelech (1999) calculated a carbohydrate need of 20 g to immobilize 1.0 g of N, based on a microbial C/N-ratio of 4 and a 50% C in dry carbohydrate.

The present study aimed to reduce the inorganic nitrogen accumulating in an extensive shrimp culture system by (1) increasing the C/N ratio of the feed through reducing its protein content and by (2) increasing the C/N ratio further through carbohydrate addition to the ponds. These manipulations should facilitate the development of heterotrophic bacteria and the related in situ protein synthesis, which in turn will contribute to the protein intake of the shrimps. Also, this study aims to reduce the inclusion level of fishmeal in the feeds of marine shrimp to enhance sustainable development of marine shrimp aquaculture in Egypt.

MATERIALS AND METHODS

Experimental design

The experiment was carried out at the Shrimp and Fish International Company (SAFICO), South Sinai, Egypt, over a 120 days (from 1st of may, 2010 to 1st of September, 2010) feeding trial in 10 greenhouses with an average area of 300 m^2 and 1.0 m average depth each. Postlarvae (PL_{15}) shrimp *Penaeus semisulcatus* were purchased from (Haraz's Marine Fish and Shrimp Hatchery, El- Qantara, Ismailia). Shrimp were grown in a nursery to an initial stocking weight of 1.55 ± 0.2 g for the experimental feeding trial. The feeding experiment consisted of four treatments (with two replicates) and one control (with two replications) was compared. In control greenhouses, the shrimp were fed with artificial feed with 42.95% crude protein (CP), whereas in the bioflocs treatments, the shrimp were fed with bioflocs and artificial feeds with different CP%. The description of the different treatments and control used is as follow:

1. Control: artificial feed with 42.95 CP%.
2. Treatment A 31.15 CP% ($BFA_{31.15\%}$).
3. Treatment B 21.60 CP% ($BFB_{21.60\%}$).
4. Treatment C 18.45 CP% ($BFC_{18.45\%}$).
5. Treatment D 16.25% CP% ($BFD_{16.25\%}$).

Feeds

A control feed (high protein content) in the absence of biofloc production was challenged against four feeds formulated with different levels of crude protein (low protein diet) in the presence of biofloc production. The basic guideline for the formulation of the feeds was to reduce the inclusion of fishmeal. Different CP levels in all feeds were achieved by manipulating various inclusion levels of fishmeal protein. All feeds were produced at the feed preparation section of the study farm.

The experimental feeds were analyzed for the proximate composition following Association of Analytical Chemist Methods (A.O.A.C., 2000). Moisture content was determined by drying in an oven at

85°C to constant weight. Crude protein was determined indirectly from the analysis of total nitrogen (crude protein = amount of Nitrogen x 6.25) using Kjeldahl method while crude lipid was determined after soxhlet extraction of dried samples with 1.25% H_2SO_4 and 1.25% NaOH. Ash content after ashing in a porcelain crucible placed in a muffle furnace at 550°C for 16 hours. Proximate compositions of experimental feeds were presented in Table 1.

Feeding trial

At the start of the experiment, the shrimp had an average weight (± standard deviation) of 1.55 ± 0.2 g. The rearing units consisted of 10 greenhouses and were stocked at an initial stocking density of 30 individuals /m^2. Feed was given at 5% of the shrimp biomass. Feed were given in daily four meals. Wheat flour was also added at a rate of 50% of feed applied to each biofloc treatment to maintain an optimum C:N ratio for bacteria (Goldman *et al*, 1987; Avnimelech, 1999 and Hargreaves, 2006). Wheat flour was spread to the greenhouses surfaces in the afternoon and completely mixed with the water of each greenhouse by strong aeration system. Discharged water from the farm of the present study was used as inoculum to develop the biofloc. Nutritional value of biofloc was determined every 30 days in order to obtain information on its nutritional contribution to each biofloc treatment.

Table 1. ***Proximate composition of formulated feeds based on dry weight basis (g/100g)***

Constituent	Test diets				
	Control	BFA 31.15	BFB 21.60	BFC 18.45	BFD 16.25
Crude protein (%)	42.95 ± 1.06	31.15 ± 0.77	21.60 ± 0.53	18.45 ± 0.45	16.25 ± 0.40
Crude fat (%)	20.18 ± 0.86	13.21 ± 0.97	14.56 ± 0.73	16.47 ± 0.93	14.47 ± 0.85
Crude fiber (%)	3.60 ± 0.07	2.29 ± 0.04	2.86 ± 0.06	3.82 ± 0.07	2.28 ± 0.04
Total ash (%)	18.54 ± 0.72	10.23 ± 0.55	9.08 ± 0.64	10.46 ± 0.82	9.29 ± 0.74
Moisture (%)	5.7 ± 0.83	6.9 ± 0.91	7.9 ± 0.88	7.8 ± 1.13	7.8 ± 0.92
Carbohydrates* (%)	9.03	36.22	44.00	43.00	49.91

***Carbohydrates calculated by difference.**

The biofloc was collected from each treatment using a net and was dried in an oven at 85°C to constant weight. The amount of CP% was determined by the (A.O.A.C., 2000) methods, lipid% were estimated by the Bligh and Dyer (1959) Method as modified by Kates (1986). Total n-3 PUFA (mg/g DW) and total n-6 PUFA (mg/g DW) were determined using the NOAA protocol (1988) of National Marine

Fisheries for the analysis of marine fish oil.

Shrimp performance indicators

Weekly sampling to estimate survival rate %, Feed conversion ratio (FCR), average final body weight (ABW), weight gain (g $week^{-1}$) were used to assess dietary effects on shrimp performance. At the end of the 120-days experiment, the same performance indicators were estimated in addition to final yield (kg shrimp $pond^{-1}$) per treatment basis.

Economic Analysis

The economic analysis was computed to estimate the cost of feed required to raise a kilogram of shrimp using the various experimental feeds. The major assumption is that all other operating costs for commercial shrimp production will remain the same for all feeds. Thus cost of feed was the only economic criterion in this case. The cost was based on the current prices of the feed ingredients as at the time of purchase. The economic evaluations of the feeds were calculated from the method of (New 1989 and Mazid *et al*., 1997) as:

Estimated Investment cost analysis = Cost of Feeding (L.E) + Cost of stocked shrimp postlarvae (L.E).

Profit Index = Value of shrimp cropped (L.E)/Cost of Feed (L.E).

Net Profit = Total value of shrimp cropped (L.E) – Total Expenditure (L.E).

Benefit: Cost Ratio (BCR) = Total value of shrimp cropped (L.E) / Total Expenditure (L.E).

Water quality

During the experimental period, water quality in the culture systems was monitored daily for dissolved oxygen (mg/L), salinity (ppt), pH and temperature ^{o}C in all greenhouses at sunrise (05:30 – 06:00) using a YSI 556MPS meter (Yellow Spring Instrument Co., Yellow Springs, OH,USA). Nitrite (NO_2–N mg/L) and nitrate (NO_3–N mg/L) were analyzed spectrophotometrically according to standard methods (APHA, 1998).

Statistical analysis

Statistical analysis was performed using SAS v9.2 for Windows (Cary, North Carolina, US, 2002–2004). Analysis of variance (ANOVA) was used for the data analysis. Tukey's HSD was employed to check for differences between means according to the method described by Zar (1996). The 5% significance level was used for all tests.

RESULTS

Biofloc consumption

The shrimp in the bioflocs treatment were actually able to consume the flocs. This was shown by grazing behavior observed in the ponds and biofloc harvested materials with shrimp samples and also by the color of their digestive tract.

Shrimp performance indicators

As presented in Table 2, no differences were observed (P>0.05) between survival rate % (85.7% to 88.4%). The development of the Biofloc enhanced the shrimp growth. Tukey's HSD revealed that all four biofloc treatments significantly outperformed the control in terms of final ABW (g), yield (kg shrimp pond^{-1}), FCR, weight gain (g week^{-1}) at harvest confirming the utilization of biofloc by fish as food.

Economically speaking, as can be seen from economic parameters in Table 3, the biofloc treatment succeeded to reduce the cost compared to the control diet. The highest feed cost was for the control feed and the lowest feed cost was for the BFD$_{16.25\%}$treatment. The best net profit of 5,785.76 was recorded from shrimp fed BFC$_{18.45\%}$ and the best Cost benefit ratio of 3.99 were recorded from shrimp fed BFD$_{16.25\%}$. This due to the higher cost of high protein shrimp feed in control diet compared to other dietary treatments in the presence of the biofloc formation. The total revenue from the harvested shrimp was higher in the dietary treatments with low CP levels in the presence of biofloc than in control diet due to the combined effect of better yield, less cost and high price of shrimps according to their marketable size (Tables 2 and 3).

Nutritional value of bioflocs

There were no significant differences (P>0.05) in the CP%, total lipids, total n-3 PUFA (mg/g D) and total n-6 PUFA (mg/g DW) between biofloc treatments (Table 4).

Water quality

The average value of dissolved oxygen, nitrate, nitrite, TAN, pH, temperature and salinity during the feeding experiment are displayed in Table 5.

Table 2. ***Mean production parameters (mean ± SD) of shrimp P. semisulcatus after 120 days of culture and fed four feeds with varying levels of CP% with biofloc grown with wheat flour as a carbon source and control feed without biofloc (42.95 g/100g).***

Parameters	Treatment				
	Control	BFA$_{31.15\%}$	BFB$_{21.60\%}$	BFC$_{18.45\%}$	BFD$_{16.25\%}$
Initial ABW (g)	1.55±0.2	1.55±0.2	1.55±0.2	1.55±0.2	1.55±0.2
Initial biomass stocked (kg/pond)	13.95	13.95	13.95	13.95	13.95
Final ABW (g)	15.1±1.6^{b}	19.8±0.1^{a}	19.2±0.3^{a}	20.1±0.3^{a}	19.0±1.1^{a}
Final yield (kg shrimp pond^{-1})	114.1±5.3^{b}	151.5±7.9^{a}	152.6±7.4^{a}	155.5±6.9^{a}	153.6±8.4^{a}

FCR	3.1±0.5[b]	1.21±0.2[a]	1.14±0.3[a]	1.11±0.3[a]	1.19±0.5[a]
Weight gain(g week^{-1})	0.85±0.4[b]	0.98±0.1[a]	1.00±0.2[a]	1.11±0.2[a]	1.00±0.2[a]
Survival rate %	86.6± 16.7	88.4± 4.3	86.9± 12.6	86.2± 9.8	85.7± 7.1

Mean values in same row with different superscript differ significantly (P<0.05).

Table 3. *Economic indices calculated from different feeding options used in the present study.*

Parameters	Treatment				
	Control	BFA$_{31.15\%}$	BFB$_{21.60\%}$	BFC$_{18.45\%}$	BFD$_{16.25\%}$
Initial biomass stocked (kg)	13.95	13.95	13.95	13.95	13.95
Final yield (kg shrimp pond^{-1})	114.1	151.5	152.6	155.5	153.6
Total amount of feed used (kg)	310.46	166.43	209.36	157.12	166.18
Price (L.E[a])/kg feed	6.00	4.00	3.00	2.00	1.50
Total shrimp PLs cost (L/E[a])	675.00	675.00	675.00	675.00	675.00
Total feed cost (L.E[a])	1,862.76	665.72	628.08	314.24	249.27
Other expenses incurred (L.E[a])	1,000	1,000	1,000	1,000	1,000
Investment cost (feeding+ shrimp PLs)	2537.76	1340.72	1303.08	989.24	924.27
Total expenditure (L.E[a])	3,537.76	2,340.72	2,303.08	1,989.24	1,924.27
Price (L.E[a])/kg shrimp	50.00	50.00	50.00	50.00	50.00
Total value of shrimp produced (L.E[a])	5,705	7,575	7,630	7,775	7,680
Net profit (L.E[a])	2,167.24	5,234.28	5,326.92	5,785.76	5,755.73
Profit Index	3.06	11.73	12.14	24.74	30.80
Benefit : Cost ratio	1.61	3.23	3.31	3.90	3.99

[a] 1.00 USD = 5.79150 EGP

Table 4. *Nutritional value of bioflocs (mean ± SD) produced in shrimp feeding experiment with wheat flour as a carbon source.*

Parameters	Treatment			
	BFA$_{31.15\%}$	BFB$_{21.60\%}$	BFC$_{18.45\%}$	BFD$_{16.25\%}$
*Crude Protein (%)	20.0 ± 5.1[a]	19.9 ± 5.3[a]	20.1 ± 5.4[a]	19.8 ± 5.3[a]
*Lipid (%)	11.9 ± 3.2[a]	11.9 ± 3.1[a]	11.8 ± 2.9[a]	11.6 ± 2.7[a]
Total n-3 PUFA (mg/g DW)	0.9 ± 0.3[a]	0.9 ± 0.3[a]	0.69 ± 0.1[a]	0.69 ± 0.1[a]

Total n-6 PUFA (mg/g DW)	23.0 ± 13[a]	23.0 ± 13[a]	22.9 ± 14[a]	22.6 ± 17[a]

Mean values in same row with different superscript differ significantly (P<0.05).
[*]*Analysis based on dry weight basis (g/100g)*

There were no significant differences (*P*>0.05) in the pH, water temperature and salinity between BFT and control greenhouses. The concentrations of nitrogen species (nitrate, nitrite and total ammonia –N (TAN)) in the control greenhouses was significantly (*P*<0.05) higher than that of other treatments. There was a significant difference (*P*<0.05) in dissolved oxygen between control and all other BFT treatments. The addition of carbohydrate to the water column (*P*<0.05) reduced the nitrate, nitrite-N levels and TAN in the experimental greenhouses. The ANOVA results showed that the protein level in the diet was having a significant effect (*P*<0.05) on the water TAN, nitrite-N and total nitrogen concentrations, as can be seen from different dietary treatments. There was a significant differences (*P*<0.05) between BFT ponds and the control ponds (Table 5).

DISCUSSION

Biofloc: A sustainable solution for reduction of feed cost

Microbial flocs produced in this study could offer the shrimp industry a novel alternative feed and reduction in the dependency on fish oil and fishmeal in feeding marine shrimp. In this study, microbial flocs were produced in intensive shrimp greenhouses using wheat flour as a carbon source. Feed was applied at 5% of the total shrimp biomass in daily four rations. The nutritional quality of biofloc was appropriate for shrimp (Table 4). There was significant difference (*P*<0.05) in shrimp growth/production between control and biofloc treatments of varying low protein levels.

Survival and abnormality were compared and no significant differences (*P*>0.05) between BFT and control diet indicating no increased shrimp stress due to the presence of

Table 5. ***Summary of water quality parameters observed over 120-days growing period in the shrimp culture system during the feeding experiment.***

Treatment	Parameters						
	Dissolved oxygen (mg/L)	Nitrate-N (mg/L)	Nitrite-N (mg/L)	TAN (mg/L)	pH	Temperature °C	Salinity (ppt)
Control	5.80±2.43[c]	1.40±0.78[d]	0.1±0.23[d]	1.05±1.51[c]	8.07±0.86[a]	28.6±1.6[a]	44.1±1.3[a]
$BFA_{31.15\%}$	6.57±2.32[b]	1.20±0.74[c]	0.08±0.19[c]	0.71±1.07[b]	8.11±0.83[a]	28.9±1.6[a]	44.3±1.2[a]
$BFB_{21.60\%}$	7.59±2.48[a]	0.90±0.76[b]	0.06±0.17[b]	0.69±1.07[b]	8.21±0.83[a]	28.8±1.5[a]	45.0±1.5[a]
$BFC_{18.45\%}$	7.60±2.42[a]	0.60±0.73[a]	0.05±0.15[a]	0.53±1.05[a]	8.05±0.87[a]	29.1±1.7[a]	45.0±1.3[a]
$BFD_{16.25\%}$	7.63±2.39[a]	0.57±0.71[a]	0.05±0.15[a]	0.51±1.04[a]	8.29±0.85[a]	28.9±1.5[a]	44.5±1.3[a]

Mean values in same column with different superscript differ significantly ($P<0.05$).
Values are (Mean ± SD).

biofloc. Overall shrimp growth and production was good in terms of commercial feasibility (Table 3).

During the feeding experiment, it can be seen that the shrimp in all the biofloc treatments were able to consume the flocs. This was visually observed by the color of the digestive tract of the shrimp. The shrimp fed with control feed showed greenish digestive tracts similar to the color of the feed whereas those fed with bioflocs revealed whitish and brownish digestive tracts, similar to the colors of the bioflocs.

There was no significant difference ($P>0.05$) in survival between the control and all other treatments. The ABW of biofloc treatments was significantly ($P<0.05$) higher than control and there was no significant difference ($P>0.05$) among biofloc treatments. Based on visual observation made during the experiment, the shrimps in control diet reduced feed intake which showed by sampling, checking feeding trays and by observing the empty digestive tract. This can be due to several reasons such as the palatability of feed, stress due to disease infection or water quality deterioration. Also, Tacon (1987) found that the absence of feed attractant and low palatability may also have been the cause of less feed consumption in control diet.

During the culture period, the biofloc treatments showed good floc formation. Crab *et al*. (2007) pointed out that at moderate mixing rate as practiced in aquaculture system (1 – 10 W/m^3), microbial cells in permeable aggregates grow better than single dispersed cells due to higher accessibility to the nutrients. Intense aeration on the other hand minimizes the advantage of growing in flocs and free cells show a higher nutrient uptake.

The differences in growth when the biofloc was included were evident. Survival rates did not vary (P>0.05) among dietary treatments; however, shrimp growth was significantly improved (P<0.05) for shrimp fed microbial flocs (Table 2). Eventhough numerous studies have reported enhanced survival, health, and growth rates of shrimp raised in ponds with high activity of algae, microbial flocs, and other natural biota (Avnimelech, 1999; Moss *et al*., 2000; Moss *et al*., 2001; Tacon *et al*., 2002; Burford *et al*., 2004; Cuzon *et al*., 2004; Izquierdo *et al*., 2006 and Wasielesky *et al*., 2006).

There was no abnormality or disease symptoms observed in control and biofloc treatments. Growth of shrimp as well as other aquatic organisms is mostly affected by water quality (Smith *et al*., 2000), culture systems (Williams *et al*., 1996 and Tacon *et al*., 2002), nutrition (Chen *et al*, 2006) and health condition (Rengpipat *et al.,* 1998; Rodriguez and Le Moullac, 2000 and Argue *et al*., 2002).

The bacterial protein and new cells (single-cell protein) synthesized by the heterotrophic bacterial population are utilized directly as a feed source by the cultured fish and shellfish species, thus lowering the demand for supplemental feed protein (Avnimelech, 1999). Hari *et al* (2004) reported that *Penaeus monodon* could effectively utilize the additional protein derived from the increased bacterial biomass as a result of carbohydrate addition. Burford *et al* (2004) suggested that "flocculated particles" rich in bacteria and phytoplankton could contribute substantially to the nutrition of the *Litopenaeus vannamei* in intensive shrimp ponds. Bacterial flocculation was observed, probably supporting filtering out by the shrimp and thus supplying protein that was available and suitable for shrimp nutrition.

With regard to the protein requirement of white shrimp, Kureshy and Davis (2002) reported that 32% protein diet gave a better growth of juvenile and subadult *Litopenaeus vannamei* as compared to diets with a lower (16%) and a higher (42%) protein content. It was found that the addition of carbohydrates, essentially changing the high CP % protein feed material to low CP% protein feed, led to significant reduction of inorganic nitrogen accumulation; increased utilization of protein feed; and significant reduction of feed expenditure (Avnimelech *et al*., 1992 and 1994).

In terms of shrimp growth and feed utilization, it can be seen that shrimp growth was better in the low CP treatment, most likely due to the lower concentrations of toxic inorganic nitrogen species. In addition to a lower feed conversion ratio (FCR). According to Avnimelech (1999), the protein conversion ratio (PCR) was markedly reduced in the 20% protein treatment. The PCR in the conventional 30% protein feed treatment was 4.35–4.38, meaning that only 23% of the feed protein was recovered by the fish. The PCR in the low CP% was twice as high. The increased protein utilization is due to its recycling by the microorganisms. It may be said that the proteins are eaten by the fish twice, first in the feed and then harvested again as microbial proteins. It is possible that protein recycling and utilization can be further increased.

It is not known exactly how microbial flocs enhance growth. Izquierdo *et al*. (2006) suggested lipid contributions of microbial flocs are important. It is also speculated that microbial flocs are probiotics (Bairagi *et al.,* 2002 and 2004 and Kesarcodi-Watson *et al*., 2008). Ultimately, more work needs to be done in order to fully understand the contributions of microbial flocs and natural organisms found in ponds.

There were no significant differences ($P>0.05$) in the total CP%, total lipids, total n-3 PUFAs and total n-6 PUFAs among biofloc treatments. This may be caused by the similar composition of the microbiota in the bioflocs (e.g. marine microalgae).

As shrimp aquaculture is expected to continue to increase in the coming years, shrimp prices are likely to continue to fall as production exceeds demand, therefore challenging the profitability of this industry. One factor considered to reduce shrimp production costs and increase producers profitability, is the use of feeds with low levels of fishmeal and high levels of less expensive, high quality plant protein sources. Commercial shrimp feeds are commonly reported to include fishmeal at levels between 25% and 50% of the total diet (Tacon and Barg, 1998 and Dersjant-Li, 2002). However, recent studies have shown that commercial shrimp feeds containing 30–35% crude protein can include levels as low as 7.5–12.5% fishmeal without compromising shrimp performance (Fox *et al*., 2004). Protein levels in *L. vannamei* diets have been reduced from the reported protein requirements of 30% to 44% (Guillaume, 1997) to 22% in high-intensity, zero-exchange ponds, based on the theory that the flocculated particles in these systems contribute substantially to shrimp nutrition (Hopkins *et al*., 1995 and McIntosh and Avnimelech, 2001).

Due to the fact that proteins are the expensive component of the feed, its reduction was reflected in the total feed cost which decreased from 1,862.76 L.E for control diet to 249.27for the lowest protein feed $BFD_{16.25\%}$ (Table 3). On the other hand, low CP% led to significant reduction of inorganic nitrogen accumulation and improved shrimp growth.

The cost of production and the benefits positively favored all BFT treatments since the values computed are > 1.0 and the best values were for all treatments with biofloc which shows an increase in the shrimp value above the amount invested. This achieves high profit for the farmers when $BFD_{16.25\%}$ is used to reduce the inclusion level of fishmeal in the feeds of *P. semisulcatus*. This aquaculture utilization will promotes sustainable aquaculture in Egypt and helps in the attraction of new investments in the field of marine shrimp aquaculture in Egypt.

Production results obtained in this study are within the range of commercial shrimp production in intensive production systems. This study supports the theory that natural biota can provide a nitrogen source for shrimp, and that flocculated particles are likely to be a significant proportion of this nitrogen source.

If this biofloc technology proved to be successful, it could offer the shrimp industry a new culture option. A very significant further justification is the need to have alternative lower cost feeds replacing marine fish and shrimp meals. For these reasons, this study investigated if it would be possible to produce microbial floc as a potential ingredient for reducing fishmeal in shrimp feeds.

Biofloc: A sustainable solution to control water quality and environmentally friendly option

The accumulation of the nitrogen species (nitrate, nitrite, and TAN) in the culture systems is due to the decomposition of uneaten feed and excretion products. The nitrogen species concentration in control was significantly (P<0.05) higher than other treatments. This suggested that nitrification was likely to occur in this treatment. In the biofloc treatments, the nitrification bacteria were possibly outcompeted by the heterotrophic bacteria. The presence of high TAN concentration in the control indicated that there was not sufficient organic carbon available to convert all inorganic nitrogen into bacterial biomass compared to all other biofloc treatments.

Intensification of aquaculture systems is inherently associated with the enrichment of the water with respect to ammonium and other inorganic nitrogenous species. The management of such systems depends on the developing methods to remove these compounds from the pond. One of the common solutions used to remove the excessive nitrogen is to frequently exchange and replace the pond water. However, this approach is limited because environmental regulations prohibit the release of the nutrient rich water into the environment; the danger of introducing pathogens into the external water; and the high expense of pumping huge amounts of water.

The practical usage of biofloc technology is essential strategy for environmental protection due to strict legislation regarding the discharge of farm effluents into the neighboring water bodies (seas, rivers and lakes). Water quality values in the present study were considered optimal for shrimp culture (Davis *et al*., 1993; Lawrence and He, 1999; Van Wyk *et al*., 1999; Cuzon *et al*., 2004 and Fox *et al*., 2006).

Nitrogen produced in aquaculture systems is controlled by feeding bacteria with carbohydrates, and through the subsequent uptake of nitrogen from the water, by the synthesis of microbial protein. The relationship between adding carbohydrates, reducing ammonium and producing microbial proteins depends on the microbial conversion coefficient, the C/N ratio in the microbial biomass and the carbon contents of the added material (Avnimelech, 1999).

Typically, only 20–25% of fed protein is retained in the fish raised in intensive systems (Avnimelech, 2006), the remainder being lost to the system as ammonia and organic N in feces and feed residue. Microbial breakdown of organic matter leads to the production of new bacteria, amounting to 40–60% of the metabolized organic matter (Avnimelech, 1999). Under optimum C:N ratio, inorganic nitrogen is immobilized into bacterial cell while organic substrates are metabolized. The conversion of ammonium to microbial protein needs less dissolved oxygen compared to oxygen requirement for nitrification (Avnimelech, 2006 and Ebeling *et al.,* 2006) suggesting the preference of heterotrophic community rather than nitrifying bacteria in the BFT system. In addition, the growth rate and microbial biomass yield per unit substrate of heterotrophs are a factor 10 higher than that of nitrifying bacteria (Hargreaves, 2006).

The new strategy that is presently getting more attention is the removal of ammonium from the water through its assimilation into microbial proteins by the addition of carbonaceous materials to the system. This offer the potential utilization of microbial protein as a source of feed protein for fish or shrimp. The fact that relatively large microbial cell clusters are formed due to flocculation of the cells, alone or in combination with clay or feed particles (Harris and Mitchell, 1973 and Avnimelech *et al.,* 1982) additionally favors cell uptake by fish.

The conventional control means for ponds are to intensively exchange the water, a strategy that is not always practical, and to stop feeding to slow down TAN build up. The proposed method enables keeping a high biomass and to have a corrective means in case of a failure of conventional controls.

Added value of bioflocs technology for shrimp production

According to De Schryver *et al.* (2008), the added value that BFT brings to aquaculture is represented by the reduced costs for water treatment that is not needed anymore. Bioflocs do not allow for a complete replacement of the traditional food but still can bring about a substantial decrease of the processing cost since the feed represents 40–50% of the total production costs (Craig and Helfrich, 2002).

The potential savings of feed that can be obtained by BFT in shrimp culture was theoretically calculated as described in De Schryver *et al*. (2008). According to Eric De Muylder (2009), white shrimp can be produced with artificial feed with 35% CP at an average food conversion ratio (FCR) of 2.0.

In a culture without application of bioflocs technology, the FCR of 2.0 with 35% CP, which means 2 kg of feed, is required to produce 1 kg shrimp

- 2 x 0.35 = 0.7 kg protein is given per kg shrimp produced
- According to Eric De Muylder (2009), only 20% of the feed is taken up by the shrimp. Thus, 0.2 x 0.7 = 0.14 kg protein is taken up per kg shrimp produced.

In a culture system with bioflocs application, the FCR of 2.0 with 35% CP, which means 2 kg of feed, is required to produce 1 kg shrimp

Part of the feed will be recycled into flocs, which can also be consumed by the shrimps as feed source. Therefore, less artificial feed is applied to the system. Take F the amount of artificial feed added to the system if BFT is applied:

- With flocs, F kg feed is given per kg shrimp produced.
- (0.35 x F) kg protein is given per kg shrimp produced
- 0.35 x (0.2 x F) = 0.07 x F kg protein is taken up per kg shrimp produced.
- *As much as 80% of the artificial feed is thus unused and recycled into the flocs*
- (0.8 x F) kg feed is recycled
- 0.8 x 0.35 x F is recycled = 0.28 x F kg protein recycled
- Assume that the shrimp also take only 20% of the flocs
- 0.2 x (0.28 x F) = 0.056 x F kg protein is taken up out of the flocs per kg of shrimp produced

Calculation of the amount of external feed needed when BFT is applied. Total requirement of protein by the shrimp is 0.14 kg protein per kg of shrimp produced:

- Total protein requirement = protein obtained from the feed + protein from the flocs = 0.14
- Total protein requirement = 0.07 x F + 0.056 x F = 0.14
- The amount of feed that still needs to be applied = 1.11 kg

Calculation of the amount of organic carbon needed to grow the flocs

- 0.8 x 1.11 = 0.9 kg of the feed is unused per kg of shrimp produced
- 0.35 x 0.9 = 0.3 kg protein is unused per kg of shrimp produced (protein content of the feed is 35%)
- 0.16 x 0.3 = 0.05 kg nitrogen is unused per kg of shrimp produced (16% nitrogen content of protein) and is recycled into floc biomass. The flocs have a C/N ratio of 4 (Avnimelech, 1999)
- 4 x 0.05 = 0.2 kg C in floc biomass is produced per kg of shrimp produced. The yield of bacterial biomass can be taken to be 0.5 (Avnimelech, 1999)
- 0.2 / 0.5 = 0.4 kg C needs to be added to the water for the flocs to be able to assimilate the excess nitrogen per kg shrimp produced.
  - ➢ *If glycerol (40% of C) is used as carbon source*

- 0.4/0.4 = 1 kg of glycerol needs to be added to the water per kg shrimp produced.

1.1.1 Calculation of the cost saving for shrimp culture using BFT is as follow

➢ *The feed cost for the production of 1 kg shrimp without BFT*

- 2 kg feed per kg shrimp x 0.9 € per kg of feed = 1.8 € per kg shrimp produced.

- *The feed cost for the production of 1 kg shrimp with BFT*

- (1.11 kg feed per kg shrimp produced x 0.9 € per kg of feed) + (1 kg glycerol per kg shrimp produced x 0.15 € per kg glycerol) = 1.2 € per kg shrimp produced. Thus, the feed cost for the production of 1 kg shrimp with BFT is 33% less than without BFT.

Based on the theoretical and practical calculation, the application of BFT is believed to reduce the feed cost. This is in agreement to other studies (Avnimelech, 2007 and Hari *et al*., 2004 and 2006). Besides the reduction in feed cost, the application of BFT also brings other beneficial effects such as better water quality, which, leads to the more environmentally friendly aquaculture practices and increase biosecurity which can control the transmission of the aquatic animal diseases (Tacon *et al*., 2002).

CONCLUSION AND RECOMMENDATION

In conclusion, the present study suggests that *P. semisulcatus* are capable for ingesting and retaining nitrogen derived from natural biota. Based on the result, it can be concluded that biofloc technology provide a solution to reduce feed cost and minimize the environmental impacts. At low CP%, the shrimp fed biofloc showed better growth rate as that of the control. This indicates that biofloc is a sustainable strategy to reduce feed cost, environmental control and supporting shrimp farming.

Based on practical experience and results gained in this study, some recommendations are suggested:

- Other nutritional parameters of the biofloc such as amino acids profile, lipid class, vitamins and minerals content should be measured.
- The use of carbon sources with low price such as molasses, tapioca flour, rice bran, etc. should be investigated.
- The effect of biofloc on the survival and pathogenicity of *Vibrio* should be monitored.

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تأثير استخدام البيوفلوك الميكروبي على جودة المياه ، معدل الإعاشة ونمو الجمبري السويسي المغذى على علائق مختلفة في نسبة البروتين.

1: حل مستدام لمشاكل الاعتماد على استخدام مسحوق السمك ، زيت السمك ، والمشاكل البيئية.

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أجريت تجربة حقلية لتقييم تأثير التغذية بعلائق مختلفة في تركيز البروتين في وجود وفى غياب البيوفلوك على جودة المياه، معدل الإعاشة ونمو الجمبري السويسي في أنظمة استزراع الجمبري المكثف. تم تكوين ودراسة خمس علائق مختلفة في نسبة البروتين. أربع معاملات تم تغذيتها على علائق مختلفة في نسبة البروتين تحت وجود نظام البيوفلوك ومعاملة مقارنة من غير البيوفلوك في 10 صوب بمساحة 300 متر مربع للصوبة وكانت المعاملات كالتالي: معاملة بها نسبة بروتين 31.15%CP ($BFA_{31.15\%}$) ، معاملة بها نسبة بروتين 21.60% CP ($BFB_{21.60\%}$)، معاملة بها نسبة بروتين 18.45% CP ($BFC_{18.45\%}$)، معاملة بها نسبة بروتين 16.25% CP ($BFD_{16.25\%}$)، معاملة مقارنة (كنترول) مغذاة على عليقه بها نسبة بروتين خالية من البيوفلوك42.95%CP . تم إنماء البيوفلوك في المعاملات باستخدام دقيق القمح. تم تخزين 30 وحدة جمبري لكل متر مربع بمتوسط وزن 1.55 ± 0.2 g وكلا من الكنترول والمعاملات تكونت من مكررتين لكل معاملة في تجربة التغذية لمدة 120 يوما. وجدت اختلافات معنوية ($P<0.05$) بين الكنترول والمعاملات في متوسط الوزن النهائي للجمبري عند انتهاء التجربة بعد 120 يوم فكانت:

15.1 ± 1.6، 19.8 ± 0.1، 19.2 ± 0.3، 20.1 ± 0.3 و 19.0 ± 1.1 لكلا من الكنترول، $BFA_{31.15}$، $BFB_{21.60}$، $BFC_{18.45}$ و $BFD_{16.25}$ على التوالي.

بخصوص كمية الجمبري المنتجة من كل معاملة تغذية، فانه وجدت اختلافات معنوية ($P<0.05$) بين الكنترول والمعاملات الأخرى عند الحصاد، فكانت:

114.1 ± 5.3 ، 151.5 ± 7.9 ، 152.6 ± 7.4 ، 155.5 ± 6.9 و 153.6 ± 8.4 لكلا من الكنترول، $BFA_{31.15}$ ، $BFB_{21.60}$ ، $BFC_{18.45}$ ، $BFD_{16.25}$ على التوالي. وجدت اختلافات معنوية في معامل التحويل الغذائي بين الكنترول والمعاملات الأخرى ($P<0.05$) . وجدت اختلافات معنوية ($P>0.05$) في معدل النمو الاسبوعي فكانت معدلات النمو في الأسبوع للكنترول والمعاملات كالتالي:

0.85 ± 0.4 ، 0.98 ± 0.1، 1.00 ± 0.2 ، 1.11± 0.2 ، 1.00 ± 0.2 جرام/أسبوع لكلا من الكنترول، $BFA_{31.15}$ ، $BFB_{21.60}$ ، $BFC_{18.45}$ ، $BFD_{16.25}$ على التوالي. لم يتأثر معدل الإعاشة بالمعاملات الغذائية ($P>0.05$) وتراوح ما بين 85.7% و 88.4%. إضافة مصدر للكربوهيدرات إلى عمود المياه ($P<0.05$) أدى إلى خفض تركيزات النيرات، النيتريت، والنيتروجين الكلى في كل الصوب المستخدمة في التجربة. وجدت اختلافات معنوية ($P<0.05$) في تركيز النترات، النيتريت و النيتروجين الكلى فى مياه الصوب بين المعاملات والمقارنة. أظهرت الحسابات الاقتصادية البسيطة أن اقل المعاملات في نسبة البروتين $BFD_{16.25}$ كانت اقل المعاملات تكلفة في إنتاج كيلوا جرام من الجمبري. بالاضافة إلى تحسن جودة المياه ونمو الجمبري مقارنة بمعاملة الكنترول مرتفعة نسبة البروتين. نتائج التحليل الاحصائى توضح أن المعاملات الموجود بها بيوفلوك كانت أفضل من معاملة الكنترول من حيث متوسط وزن الفرد عند نهاية التجربة ، كمية الجمبري المنتجة من كل صوبة، معامل التحويل الغذائي، زيادة وزن الجسم /أسبوع ، معايير جودة المياه مع عدم وجود اختلافات في معدل الإعاشة.