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EFFECT OF PROBIOTICS AND CARBON SOURCES ON GROWTH PERFORMANCE AND SURVIVAL OF INDIAN WHITE SHRIMP (*FENNEROPENAEUS INDICUS)* UNDER BIOFLOC TECHNOLOGY

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SUMMARY

The aim of this study was to determine the influence of different sources of carbon with and without addition of probiotics on growth performance, and feed utilization of Indian white shrimp (*Fenneropenaeus indicus*), in addition to water quality of biofloc production system. Control diet (35% CP), in addition to eight treatments based on partial and complete substitution of artificial feeds were used. The biofloc used in the treatments were collected from different reactors with different carbon source (Corn flour and Barley flour) with and without probiotics addition. The experiment lasted for 28 days. At the end of the experiment, the results indicated that there were significant differences between treatments in growth performance and feed utilization. The highest survival was shown by the shrimp fed with barley flour fed biofloc without probiotics and control treatments (100 ± 0.0%), while the lowest survival was shown by the shrimp fed with corn flour fed biofloc with probiotics (54 ± 1.0%). The highest specific growth rate was obtained in the control treatment (1.66 ± 0.02%) while, the shrimp fed with biofloc from barley flour carbon source without probiotics showed almost similar specific growth rate (1.61± 0.02) to that of the control treatment. The biofloc fed shrimp showed promising growth values indicating that the biofloc was sufficient for supporting shrimp growth under commercial farming conditions. Hence, the overall experiments allow concluding that based on its nutritional value; the biofloc offers an alternative strategy for an efficient use of nutrient input in aquaculture systems.

Keywords: *Indian white shrimp, probiotics, growth performance, feed utilization and water quality.*

INTRODUCTION

Biofloc technology is based on microbial manipulation within the aquaculture system. Biofloc particles contain beneficial bacteria like *Bacillus, Lactobacillus* (Anand *et al.,* 2014) and bioactive compounds like carotenoids (Ju *et al.,* 2008). These are known for probiotics and immunostimulant properties. Xu and Pan (2013) reported that biofloc enhanced the haemocyte count and antioxidant status of white leg shrimp, *Litopenaeus vannamei.* Similarly, biofloc improved the disease resistance in brine shrimp, *Artemia franciscana* against *Vibrio harveyi* (Crab *et al.,* 2010). It has been reported that nature of the carbon source affects the nutritional composition of biofloc (Crab *et al.,* 2010). However, there is a dearth of information with respect to carbon source and protein levels in the feed over growth performance and immune response in penaeid shrimps.

A study by Megahed *et al*. (2018), suggested that *Fenneropenaeus indicus* is capable for ingesting and retaining nitrogen derived from biofloc and the study confirmed the importance of biofloc in shrimp production and disease prevention of common pathogens in shrimp farming such as early mortality syndrome (EMS) disease affecting shrimp farming. The author concluded that biofloc technology can be considered as effective alternative to costly feeds and feed ingredients in shrimp feed.

The Indian prawn (*Fenneropenaeus indicus*, formerly *Penaeus indicus*), is one of the major commercial prawn species of the world. In recent years, the viral and bacterial diseases as well as environmental problems of water quality, have affected the shrimp farms causing pronounced economic losses (Hernández-Llamas *et al.*, 2016).

Single cell protein (SCP) has been considered as an alternative of protein source for some species of fishes because of its nutritional value and the cheap production cost. SCPs are microorganisms including unicellular algae, fungi, bacteria, cyanobacteria, and yeast (El Sayed, 1999). Single cell protein as feed can be utilized in two ways; directly from the cultured water as a part of natural food (BFT) or harvested and processed as part of feed ingredient in artificial food. In order to provide a carbon source for heterotrophic bacteria, carbohydrate can be directly added to the cultured water or incorporated into the artificial feed. To promote the bacterial growth, carbohydrate addition is carried out to adjust the C/N ratio of the system to 10 or more (Azim *et al.*, 2007; Cavalli, and Wasielesky, 2012 and Panigrahi *et al.*, 2018). The availability of carbon thus allows more efficient use of nitrogen as the excreted nitrogen will be converted in bacterial biomass, which is further consumed by the cultured species.

Probiotics are defined as dietary supplements, that contain potentially beneficial bacteria and which confers health benefits on the organisms (FAO, 2001). The natural and external microbiota are in permanent contact with the animals; considering it, Verschuere *et al.* (2000) suggested a new term of probiotic for aquaculture, which includes their effect over the microbial community of the water, and over the quality of the farming environment. Several studies indicated that the probiotics could, contribute to enzymatic digestion, take part inhibiting pathogenic microorganisms, promote growth factors as well as increase immune response (Verschuere *et al.*, 2000 and Krummenauer *et al.*, 2014).

The aim of this study was to determine the influence of two different sources of carbon with and without addition of probiotics on growth performance, feed utilization and biofloc nutritional value on Indian white shrimp (*Fenneropenaeus indicus)*, in addition to water quality of biofloc production system.

MATERIALS AND METHODS

This study was carried out at National Institute of Oceanography and Fisheries (NIOF), Egypt on *Fenneropenaeus indicus* to determine the influence of probiotics and two different sources of carbon on growth performance, feed utilization**,** biofloc nutritional value and water quality under biofloc system.

Experimental setup:

Four reactors were prepared using PVC tanks of 40 L in capacity. Influent consisted of seawater with 25 mg/L of N, 3.6 mg/L of P and some trace elements from plant fertilizer (0.1 mL/L influent). A peristaltic pump distributed the influent continuously. The hydraulic retention time was 2 days. The carbon source, Corn flour and Barley flour was added daily at 0.6 g/L and 0.9 g/L, respectively, to obtain a C/N ratio of 10:1. A probiotic mixture was added to the reactor with probiotics treatments every two weeks at a concentration of 2 x 10^6 CFU/mL. The shrimp *F. indicus* was used as the test organism. At the start of the experiment, the shrimp had an average weight of 12.5 ± 0.1 g and were stocked at an initial density of 12 individuals per tank.

The feeding experiment:

Eight different treatments with 3 replicates in addition to control were studied. In control treatment, the shrimp was fed with artificial feed with 35% DW crude protein whereas in the biofloc treatments, the shrimp fed with 100% and 50% biofloc collected from different reactors with different carbon source with or without probiotic addition.

(1) Control: artificial feed with 35% crude protein
(2) and (3) Corn flour + probiotics: biofloc with Corn flour as carbon source with probiotics addition at 50 and 100% biofloc.
(4) and (5) Corn flour: biofloc with Corn flour as carbon source without probiotics addition at 50 and 100% biofloc.

(6) and (7) Barley flour + probiotics: biofloc with Barley flour as carbon source with probiotics addition at 50 and 100% biofloc
(8) and (9) Barley flour: biofloc with Barely flour as carbon source without probiotics addition at 50 and 100% biofloc.

Feed was given at a weight of 2.5 % of the wet shrimp biomass. Half of the feed was given in the morning and the other half in the evening, while, for 50% biofloc + 50% feeds (in the biofloc treatment we used the dry matter content of 1L biofloc to calculate the amount of biofloc water added to each tank, and based on the dry matter that gives 2.5% feed of the total biomass during the experimental period). The biofloc were collected from the reactor by using a net. In order to obtain the same level of protein input in each treatment (isonitrogenous diet), the amount of biofloc given as feed was determined based on the crude protein content and the dry weight of biofloc, which was measured every 4 days. When the biofloc were too small to be collected, the biofloc were added to the shrimp tank together with the water. The amount of water with biofloc was determined based on the crude protein content and the total suspended solid (TSS) measurement.

Growth Parameters and feed utilization:

The average body weight (ABW), average body weight gain (%), survival (SR), Specific growth rate (SGR) , and Feed conversion ratio (FCR) of the shrimps were determined every week until the completion of culture. The total body weight (W) was recorded from each experimental container along with the number of live shrimps (N). The amount of feed used in each tank was recorded. The ABW was computed from W and N. The overall average values of survival (%), the growth rate of shrimp (gm/day), percentage weight gain, and feed conversion ratios (FCR) were computed as follows:

FCR = Feed applied / Body weight gain

SGR = (ln (final weight (g)) - ln (initial weight (g)) / duration of culture (days) x 100

SR (%) = number of shrimp harvested / number of shrimp stocked x 100

Proximate analysis of experimental diet and Biofloc:

The proximate composition of the experimental diets and the biofloc were determined following the standard methods of AOAC (2019). Fatty acid methyl esters (FAME) were prepared by transesterification for gas chromatography according to Coutteau and Sorgeloos (1995) and identified by a gas chromatograph equipped with temperature programmable on-column injector.

Water quality:

During the experimental period, water quality in the culture systems was monitored daily for dissolved Oxygen (mg L-1), pH and temperature ^{o}C using a YSI 556 MPS meter (Yellow Spring Instrument Co., Yellow Springs, OH,USA). Ammonia ($NH4^{+}$), Nitrite ($NO_2^{-}N$ mg L^{-1}) and Nitrate ($NO_3^{-}N$ mg L^{-1}) were analyzed spectrophotometrically (APHA, 2005).

Statistical Analysis:

The data obtained in this study were analyzed by one-way ANOVA procedure of Statistical Analysis System (SAS 2000). Means were compared by Duncan's new multiple ranges test (Duncan, 1955).

RESULTS AND DISSCUTION

Growth performance and feed utilization:

Weight of shrimp in grams and weight increment data observed weekly for different treatments is presented in Table (1). Observations on the growth during the overall study indicated that the final body weight among treatments were differed significantly ($P<0.05$).It can be seen that the control revealed a continuously increasing average final body weight during the culture period and thus having the highest growth rate. The lowest growth rate occurred in the corn flour carbon source treatment (100%biofloc) and barley flour carbon source treatment (100% biofloc) with and without adding of probiotic. The average body weights of the shrimps in this treatment tended to decrease in time. This resulted in a negative growth rate. Many studies have shown that growing of shrimp species such as *L. vannamei* in biofloc system can improve shrimp growth as compared to clear water (Khanjani *et al*., 2016 and Panigrahi *et* *al.*, 2018). Also, the results indicated that the abnormalities found in shrimp fed with biofloc with corn flour as carbon source possibly correspond with the shrimp cannibalistic behavior. Starvation, which was caused by the poor feed accessibility, likely triggered the cannibalistic behavior of the shrimp (FAO, 2003). Application of carbohydrate improves growth rate in *F. indicus* (Megahed and Mohamed, 2014; Megahed *et al.*, 2018) *L. vannamei* (Serra *et al.*, 2015) and in *P. monodon* (Anand, *et al.*, 2017) and Biofloc used as a natural food for the cultured shrimp (Burford *et al*, 2004) apart from being a source of bioactive compounds and growth promoters (Ju *et al.*, 2008; Megahed and Mohamed, 2014; Megahed *et al.*,2018). Moreover, digestive enzyme secretions from many probiotic bacteria like *Bacillus*, *Lactobacillus* in the carbohydrate added groups might have improved the shrimp growth performance (Ringo, *et al.*, 2012; Anand *et al.*, 2014; Megahed and Mohamed, 2014; Megahed *et al.* 2018). While, 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 when the substitution level of artificial feed by the biofloc was 50%, all the biofloc treatments showed a positive growth rate, yet still lower than control. The Barley flour (50% biofloc) without probiotics treatment revealed the highest growth rate when compared to the other biofloc treatment. These results agree with results obtained by Kuhn *et al.* (2008) carried out on shrimp juveniles showed that shrimp fed with 50% artificial feed (35% CP) and 50% microbial flocs was superior in performance than animals fed at the 50% artificial feed ration level In agreement with the present study.

Survival rates did significantly vary ($P<0.05$) among dietary treatments; however, The highest average survival were observed in the Barley flour treatment without probiotic and control treatment in contrast with corn flour with probiotics treatment (100% biofloc), where survival was only 54%. While the lowest survival in the corn flour treatment with probiotic (50%) was likely due to starvation because the shrimps stopped eating from the second week of culture onward. Survival of the control and Barley flour treatments was highest than in the other treatments. This results are in agreement with 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 (Cuzon *et al.*, 2004; and Wasielesky *et al.*, 2006; Megahed and Mohamed 2014 and Megahed *et al*., 2018).

Table (1): Effect of treatments on growth performance and feed utilization of shrimps (*F. indicus*) fed with artificial feed (control) and biofloc with different carbon sources (Corn flour and Barley flour) with and without probiotics addition on day 28.

Parameter	Control	Corn flour + probiotics		Corn flour		Barley flour + probiotics		Barley flour	
		100% biofloc	50% biofloc	100% biofloc	50% biofloc	100% biofloc	50% biofloc	100% biofloc	50% biofloc
Initial wt. (g)	12.5± 0.1	12.5± 0.1	12.5± 0.1	12.5± 0.1	12.5± 0.1	12.5± 0.1	12.5± 0.1	12.5± 0.1	12.5± 0.1
Final Wt. (g)	19.9 ± 0.1^{a}	13.8 ± 0.1^{g}	19.0 ± 0.1^{c}	13.9 ± 0.8^{f}	18.2 ± 0.7^{d}	13.0 ± 0.1^{h}	18.1 ± 0.7^{e}	12.7 ± 0.9^{i}	19.6 ± 0.1^{b}
Weight gain (g)	7.1± 0.001^{a}	1.3± 0.002^{e}	6.7± 0.001^{b}	1.1± 0.001^{f}	5.7± 0.001^{c}	0.5± 0.00^{g}	5.6± 0.001^{d}	0.2± 0.0^{h}	7.1± 0.001^{a}
Weight gain (%)	56.8 ±0.1^{g}	10.4± 0.1^{e}	53.6± 0.5^{b}	8.8± 0.2^{f}	45.6± 0.5^{c}	4.0± 0.4^{h}	44.8± 0.2^{d}	1.6± 0.01^{i}	56.8± 0.4^{a}
SR (%)	100 ± 0.0^{a}	54 ± 1.0^{g}	67 ± 1.0^{f}	71 ± 1.0^{e}	89 ± 1.0^{c}	82 ± 1.2^{d}	93 ± 1. 0^{b}	100 ± 1.0^{a}	100 ± 0.0^{a}
SGR (%/day)	1.66 ± 0. 02^{a}	0.35± 0. 01^{g}	1.50± 0.0^{c}	0.38 ± 0.02^{f}	1.34 ± 0.01^{d}	0.14 ± 0.3^{h}	1.32± 0.01^{e}	0.06 ± 0.0^{j}	1.61 ± 0.02^{b}
FCR	1.98± 0.02^{c}	1.89± 0.01^{d}	1.99± 0.01^{b}	1.54± 0.01^{f}	1.20± 0.002^{h}	1.20± 0.002^{h}	1.30± 0.001^{g}	3.76± 0.001^{a}	1.87± 0.001^{e}

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

Effect of probiotic and biofloc on nutritional value of biofloc:

The crude protein content of the biofloc during the experiment were significantly different ($P<0.05$), it ranged from 15 to 35 % in average (Table 2). The highest protein content (35%) and total n-3 PUFA (0.9

mg/g DW) in the control treatment whereas in barley flour carbon source with probiotic treatment at 50% biofloc (34% and 0.9), while the lowest protein content (15%) was in barley flour carbon source treatment at100% biofloc The biofloc with corn flour and probiotics addition at 100% biofloc showed the highest average total n-6 PUFA. The crude protein content of the biofloc grown with barley flour and probiotics addition showed the highest level and was significantly different (*P<0.05*) from the other treatments. This result was iIn agreement with (Megahed and Mohamed, 2014; Vargas-Albores *et al.*, 2017; Hostins *et al.*, 2017 ; Putra *et al.*, 2017 and Megahed *et al.*, 2018)

Effect of probiotic and biofloc on water quality:

Water temperature, pH, dissolved oxygen (DO), ammonia ($NH4^+$), nitrite (NO_2) and nitrate (NO_3) are shown in Table (3). No significant differences (*P > 0.05*) were observed for water temperature, pH, while $NH4^+$ and values among different carbon sources treatments with control treatment during the experimental period were significantly different. The average total amino nitrogen (TAN), ammonia, nitrite and nitrate during the experimental period are presented in Table (3). TAN of the water in the shrimp rearing tanks were significantly different (*P<0.05*). Concentrations of TAN and nitrite N, recorded during the experiment, were at optimum levels as recommended for juveniles of Pacific white shrimp *L. vannamei* (Samocha *et al.* 2004) and *F. indicus* (Megahed and Mohamed, 2014; Megahed *et*

Table (2): Effect of treatments on nutritional value of biofloc used in shrimp (*F. indicus*) feeding experiment, which grown with different carbon source with and without the addition of probiotics.

Parameter	Control	Corn flour + probiotics		Corn flour		Barley flour + probiotics		Barley flour	
		100% biofloc	50% biofloc	100% biofloc	50% biofloc	100% biofloc	50% biofloc	100% biofloc	50% biofloc
Crude protein (%)	35 ± 0.4[a]	20 ± 0.4[f]	27 ± 0.5[d]	19 ± 0.7[g]	29 ± 0.6[c]	22 ± 0.4[e]	34 ± 0.2 [b]	15 ± 0.3[h]	19 ± 0.4 [g]
Lipid (%)	8 ± 0.04[d]	11± 0.04[b]	6 ± 0.04[f]	12± 0.04[a]	7 ± 0.04[e]	9 ± 0.04[c]	5± 0.04[g]	7± 0.04[e]	4 ± 0.04[h]
Total n-3 PUFA (mg/g DW)	0.9± 0.04[a]	0.7 ± 0.03[b]	0.4 ± 0.01e	0.3 ± 0.03[f]	0.5 ± 0.01[d]	0.9 ± 0.01[a]	0.9 ± 0.01[a]	0.7 ± 0.01[b]	0.6 ± 0.01[c]
Total n-6 PUFA (mg/g DW)	23± 0.1[b]	27.6± 0.1[a]	10 ± 0.0[f]	9 ± 0.1[g]	7 ± 0.1[h]	17 ± 0.3[d]	4 ± 0.1[i]	12 ± 0.1[e]	20 ± 0.0[c]

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

Table (3): Effect of treatments on water quality in shrimp (*F. indicus*) rearing tanks throughout the experimental period.

Item	Control	Corn flour + probiotics		Corn flour		Barely flour + probiotics		Barely flour	
		100% biofloc	50% biofloc	100% biofloc	50% biofloc	100% biofloc	50% biofloc	100% biofloc	50% biofloc
Temp. (^oC)	27.10 ±0.20	27.20 ±0.20	27.00 ±0.10	27.30 ±0.10	27.00 ±0.20	27.00 ±0.10	27.10 ±0.20	27.10 ±0 10	27.30 ±0.10
PH	8.07 ±0.20	8.05 ±0.20	8.11 ±0.20	8.07 ±0.20	8.07 ±0.20	8.06 ±0.30	8.05 ±0.30	8.07 ±0.20	8.06 ±0.30
Dissolved oxygen(mgl^{-1})	5.00 [g] ±0.04	7.02 [e] ±0.04	7.32 [b] ±0.03	7.02 [e] ±0.03	7.09[d] ±0.03	6.83 [f] ±0.03	7.23 [c] ±0.03	7.00 [e] ±0.03	7.54 [a] ±0.03
Ammonia($mg\ l^{-1}$)	0.45 ±0.03[a]	0.38 ±0.03[b]	0.34 ±0.01[d]	0.37 ±0.02[c]	0.37 ±0.02 [c]	0.37 ±0.02 [c]	0.37 ±0.02 [c]	0.37 ±0.02[c]	0.37 ±0.02[c]
Nitrite($mg\ l^{-1}$)	0.80 ± 0.01[a]	0.40 ± 0.01[c]	0.30 ± 0.00[d]	0.20 ± 0.00[e]	0.30 ± 0.01[d]	0.30 ± 0.00[d]	0.50 ± 0.02[b]	0.30 ± 0.00 [d]	0.40 ±0.01[c]
Nitrate($mg\ l^{-1}$)	1.40 ± 0.20 [a]	0.99 ± 0.20 [b]	0.98 ± 0.10 [c]	0.97 ± 0.10 [d]	0.98 ± 0.20 [c]	0.97 ± 0.20 [d]	0.98 ± 0.10 [c]	0.96 ± 0.20	0.96 ± 0.20 [e]
TAN(mg/L)	1.40 ± 0.01[a]	0.90 ± 0.01[b]	0.70 ± 0.01[c]	0.70 ± 0.01[c]	0.60 ± 0.01[d]	0.70 ±0.01[c]	0.60 ± 0.01[d]	0.50 ± 0.01[e]	0.50 ±0.001[e]

Mean values in same row with a different superscript differ significantly (P<0.05) *al.,* 2018). The low concentrations of nitrite N, observed during the culture period, suggest oxidation of ammonia to nitrate (Cohen *et al.*, 2005; Megahed and Mohamed, 2014; Megahed *et al*., 2018). Studies evaluating water quality in zero exchange systems report low concentrations of ammonia and nitrite (Ray *et al.,* 2010; Vinatea et *al.,* 2010; Megahed and Mohamed, 2014; Megahed *et al.,* 2018), The nitrite concentration in the water of the control treatment was significantly higher than the rest of experimental groups. On the other hand, the nitrite concentration in the water of both corn flour treatment (100%) and barley flour treatment was significantly the lowest ($P<0.05$) one among the other treatments. These results are in agreement with Azim *et al.* (2007), who reported factor analysis on the principal ecological processes during biofloc development. This showed that the decomposition process coupled nitrification. During decomposition of organic waste both from the uneaten feed and excreted materials, TAN and CO_2 were released into the water. TAN in turn became available for nitrification. With the continuous supply of oxygen, nitrification was promoted, leading to nitrite and nitrate accumulation in water.

CONCLUSION

It could be concluded that Barley flour + probiotics at 50% biofloc was the best in terms of growth performance and feed utilization.

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تاثير البروبيوتيك والمصادر الكربونيه علي اداء النمو ومعدل البقاء للجمبري الابيض الهندي في نظام البيوفلوك

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الهدف من هذا البحث هو دراسة تأثير اضافة مصادر مختلفة من الكربون مع اضافة او عدم اضافة البروبيوتيك على أداء النمو ، والكفاءه الغذائيه للجمبري الابيض الهندى *(Fenneropenaeus indicus)* ، بالاضافة الي نوعية المياه المربي فيها الجمبري تحت نظام البيوفلوك . تم استخدام عليقة كنترول نسبة البروتين بها (35 ٪ بروتين خام) واستخدام ثمانية انظمة غذائية معتمدة علي البيوفلوك مقارنة بالمعاملة الكنترول . و استخدام مصادر كربون مختلفه (دقيق الذرة ودقيق الشعير) مع وبدون إضافة البروبيوتيك اليها بمستويين احلال (50 و 100%) بيوفلوك. استمرت التجربة لمدة 28 يومًا. درست خلالها معدلات النمو والكفاءه الغذائيه ومعدل الاعاشه للجمبري بالاضافة الي جودة المياه تحت نظام البيوفلوك.

فقد اظهرت النتائج إلى وجود فروق ذات دلالة إحصائية بين المعاملات في أداء النمو والاستفاده الغذائيه. فكان معدل الاعاشة للجمبري الابيض الهندي المغذي علي البيوفلوك الناتج من دقيق الشعير بدون اضافة البروبيوتيك هي الاعلي (100 ± 0 ٪) ، في حين أن أدنى معدل اعاشة أظهره الجمبري الابيض الهندي المغذي علي البيوفلوك الناتج من استخدام دقيق الذرة واضافة البروبيوتيك (54 ± 1 ٪).
مجموعة الجمبري التي ربيت في البيوفلوك باستخدام دقيق الشعير كمصدر كربوني بدون اضافة البروبيوتيك أظهرت معدل نمو مماثل تقريبا للمعامله الكنترول فكان معدل النمو النوعي (1.61 ± 0.02 ٪) و (1.66 ± 0.02٪) علي التوالي.
اظهر الجمبري الذي يغذى علي بيوفلوك نموًا جيدا مقارنة بالمعاملة الكنترول ، مما يشير إلى أن البيوفلوك يكفي لتوفير الاحتياجات الغذائية للجمبري المستزرع. وبالتالي ، فإن كل المعاملات توصي باستخدام البيوفلوك اعتمادا علي قيمته الغذائية ؛ وان البيوفلوك يمكن تعميمه كنظام بديل ومستدام لتغذية الجمبري والاستغلال الامثل للمغذيات الموجودة في احواض الاستزراع اثناء فترة التربية.