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Research Article

Carbohydrate Supplement Impact on Growth Performance, Bacterial Community, and Bacterial Food Quality of Whiteleg Shrimp (*Litopenaeus vannamei*) under Biofloc System

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Biofloc technology has a high impact on enhancing shrimp production. Suitable supplemented carbohydrates (CHO) could affect the type of microorganisms developed in the system, which reflects on shrimp production, food safety, and public health. Here, we aimed to compare the effects of sugarcane molasses and wheat flour as carbohydrate sources on biofloc technology. That was achieved through measuring the following parameters: water quality, growth performance, feed utilization, floc composition, shrimp whole body composition, microbial community, and biofloc shrimp food bacterial quality. Postlarvae of whiteleg shrimp (*Litopenaeus vannamei*) with a mean weight (38.47 ± 5.8 mg) were stocked at a density of 200 individuals/m^2 and cultured under a biofloc system for 128 days in six tanks with a total water volume of 30 m^2 each. Water quality analysis revealed a better-dissolved oxygen concentration (5.59 mg/L) in the wheat flour treatment, whereas no significant differences were found between the two treatments in ammonia, nitrite, and pH levels. Increased turbidity (64.27 NTU) and floc volume (18.40 mL/L) were recorded with molasses treatment. Growth performance including final weight, weight gain, average daily gain, weight gain per week, and specific growth rate (12.37 g, 12.34 g, 0.096 g/d, 0.68, and 4.70%, respectively) were all significantly higher in the molasses treatment. Wheat flour treatment was associated with a higher survival rate (99%), biomass (71.16 Kg), and biomass increase percentage (395.337) in shrimps. It also improved feed utilization in terms of a lower feed conversion ratio (1.37) and higher protein efficiency ratio (1.92). The chemical composition of biofloc and shrimp whole body were both nutritious higher in wheat flour treatment. In water, total heterotrophic bacterial counts with sugarcane molasses treatment and wheat flour were estimated as 3.4×10^5 CFU/mL and 1.2×10^5 CFU/mL, respectively, with no significant difference. In both treatments, beneficial bacteria such as lactic acid bacteria and *Enterobacter cloacae* were identified in water with the absence of pathogenic *Vibrio* spp. Wheat flour had a significantly lower total *Vibrio*-like count (TVC). Shrimps had lower TVC (1.9×10^4 CFU/g) with flour than with molasses (1.32×10^5 CFU/g). *Cronobacter* spp. were associated with shrimps in BFT supplemented with molasses, which might pose a potential risk to food safety. In conclusion, the use of wheat flour was the best for shrimp production and shrimp food bacterial quality.

1. Introduction

High demand and economic value of crustaceans in both national and international markets have been reported, especially shrimp [1]. Although shrimp rearing has been practiced for decades, there are still many challenges to improving the shrimp culture industry [2, 3]. Biofloc technology has a high potential to face these challenges, therefore, enhancing shrimp production [4–6].

Carbon source supplementation to biofloc system technology (BFT) could flourish heterotrophic bacteria and adjust nitrogen compounds in water that maintain good water quality without water exchange [7]. Studies have confirmed that bioflocs are a good protein source for shrimp,

and it decreases the demand for protein feed as well [8, 9]. Avnimelech et al. [10] proved that carbohydrate addition can increase protein utilization and provide essential vitamins and lipids for shrimp growth. Diverse carbon sources are used to encourage microbial development in biofloc systems such as highly soluble matters like molasses, glycerol, and glucose. In addition to complex structures such as bran, flour, and starch [11–13], the immunity of cultured shrimp and the overall production could also vary according to the types of carbohydrates (CHO) [4, 6, 14–17]. Carbohydrates could reflect on biofloc nutritional values, the type of microorganisms developed in the system as well as shrimp growth performance [15, 16, 18, 19]. A suitable CHO is crucial for a successful biofloc technology.

Biofloc supplemented with organic carbons could promote the dominance of heterotrophic bacterial communities [20, 21]. The bacterial quality of shrimps raised in the biofloc technology system is a challenge, due to the possible presence of a high load of total bacterial count that may exceed the permissible standard level. High stocking densities in biofloc technology with no water exchange resulted in large amounts of organic material from feces and nonutilized feed accumulation. Consequently, a load of heterotrophic bacteria in recirculating water and shrimp increase with time [22].

Vibrio spp. are ubiquitous bacteria in seawater that might constitute up to 40% of the bacterial community [23]. They are also considered part of the natural microflora of fish and shellfish [24]. Other species of vibrios such as *V. parahaemolyticus* and *V. vulnificus* are pathogenic for humans that cause seafood-borne illnesses [25–27]. Seafood foodborne outbreaks in humans have been reported worldwide [28–31].

Supplemented carbohydrates (CHO) could enhance shrimp production, total bacterial count, and the type of microorganisms developed in the BFT. Adversely, these might reflect on shrimp bacterial quality. This work is aimed at improving BFT systems by using sugarcane molasses and wheat flour as CHO sources, subsequently, comparing their effects on water quality, growth performance, feed utilization, floc composition, shrimp whole body composition, and microbial community, and also, examining the reflection of previous factors on shrimp bacterial quality.

2. Materials and Methods

2.1. Shrimp Farming. This study was performed at a marine shrimp hatchery (brood stock section, Damietta, Egypt) from May to September 2021. It was conducted in six uniform-sized cement tanks (dimensions: $3 * 10 * 1.2$ m) with 36 m^3 total volume each filled with 30 m^3 of sand-filtered seawater (salinity 32).

2.2. Carbohydrate Source. Sugarcane molasses and wheat flour were bought from local markets. They were utilized as treatments of carbohydrate sources, each in triplicate. Shrimps were cultured for 128 days. Theoretical adding of carbon sources was performed once a day based on the calculation as described by Avnimelech [32]. The preweighed carbon sources were completely mixed with tank water and equally distributed over the tank's surface directly. The proximate compositions of study feed and carbohydrate sources are given in Table 1.

Table 1: Proximate analysis of study feed and different carbon sources used in the study.

Constituent	Feed	Sugarcane molasses	Wheat flour
Crude protein	38.15	6.73	10.33
Ether extract	10.27	3.91	3.44
Crude fiber	4.71	3.78	5.25
Total ash	8.92	17.3	1.72
Moisture	7.92	27.87	10.83

2.3. Experimental Management. Postlarvae of *L. vannamei* shrimp with a mean body weight of 38.47 ± 5.8 mg were obtained from the marine shrimp hatchery and stocked at the density of 200 shrimps/m^2. The tanks were conditioned with continuous aeration and a 12 h/12 h dark/light regime. Cultural water was continuously aerated by the installation of a web of air stones at the bottom of each tank, which was attached to aeration pipes (2 inches) and a regulator to control the air pressure in all tanks.

Shrimp were fed four times a day at 7 AM, 10 AM, 1 PM, and 3 PM with 38% protein feed (Skretting, Egypt). Daily feeding rates were 15% of shrimp body weight at the beginning of the study and then gradually decreased to 2.5% in the latest period. The feed amount was adjusted fortnightly after weighing the shrimp sample and summation of any mortality. Crumbled (0.4-0.6 mm) and pelleted feeds (0.8–1.5 mm) were used for feeding throughout the study. After feeding, molasses and wheat flour were added to their respective treatments to maintain an input C : N ratio of 15.

2.4. Water Quality. During the study time frame, water temperature, dissolved oxygen (DO), pH, ammonia (NH3), nitrite (NO2), turbidity, and biofloc volume were all monitored. Dissolved oxygen and temperature were measured using an electronic probe (HANNA, HI9146-04), pH was measured using a portable pH meter (Milwaukee, MW102), and ammonia and nitrite were both measured using a photometer (HANNA, HI97715, and HI97708, respectively), turbidity was monitored with turbidity meter (Lovibond, TB211 IR) while floc volume was measured using Imhoff cone. Water temperature and salinity were adjusted to $27.7\,\text{Co} \pm 1.85$ and 32.5 ± 0.5, respectively, during the study. There was no water exchange during the study period.

2.5. Data Collection and Analysis. The number of shrimps at the beginning of the study and the end of the study were recorded to calculate the total survival rate. Shrimps in all tanks were collected and weighed at the end of the study to measure the growth performance and feed utilization. Growth performance parameters were evaluated as final weight (FW), weight gain (WG), average daily weight gain (ADWG), weight gain per week (G/W), specific growth rate% (SGR%), final biomass, and percentage of biomass

increase. Feed utilization was estimated in terms of feed conversion ratio (FCR), feed efficiency (FE), and protein efficiency ratio (PER) [9].

As for the proximate composition of shrimps' whole body and flocs, samples were analyzed for the proximate composition according to the methodology reported by AOAC [33]. Biofloc samples were collected for biochemical analysis at the end of the study from each tank using a 100 mm mesh. Shrimp samples from all tanks were collected during harvesting for biochemical analysis. Samples were dried in a heated oven at 60°C and then grounded. For moisture contents, a known quantity of samples was dried in a heated oven at 105°C until a constant weight was obtained. Regarding the ash contents, a known quantity of dry samples was burnt in a muffle furnace at 550°C for four hours, and the ash was cooled and weighed. The crude protein content was determined by the Kjeldahl method (FOSS, KjelTecTM 8400), and crude lipid was determined by the automatic fat extraction system (FOSS, SoxtecTM 8000) and crude fiber by automatic fiber analysis system (FOSS, FibertecTM 8000). The nitrogen-free extract was estimated by the difference [34].

2.6. Bacterial Community Assessment. Water and shrimps were collected from all tanks at the end of the study. A total of 50 randomly selected shrimps (*Litopenaeus vannamei*) and one water sample from each tank (6 total) (100 mL each). Flour, molasses, and feed samples were also analyzed. Samples during collection were placed in sterile bags, placed in a cool polystyrene box containing sterile ice packs that kept the temperature at 4-6°C during transportation. Samples were cautiously transported to the Suez University laboratory and analyzed instantly.

2.6.1. Bacterial Analysis. Shrimp samples were processed in complete aseptic condition. Shrimps were deheaded and chopped into small pieces using sterile knives and forceps and placed on a sterile tray. Samples (5 g) were homogenized for 2 min in a sterile bag containing 45 mL of buffered peptone water (0.1%) (Lab M, UK), using a stomacher (Seward Stomacher 400 circulator, UK). Water samples (1 mL) were vortexed/2 min in a 15 mL screw-capped sterile tube containing 9 mL of buffered peptone water (0.1%) (Lab M, UK). Flour, molasses, and feed samples (5 g each) were homogenized for 2 min in a sterile bag containing 45 mL of buffered peptone water.

2.6.2. Heterotrophic Bacterial Count. Tenfold serial dilution was done for the total bacterial count. Dilutions up to 10^5 were spread plated onto plate count agar (PCA, Oxoid, UK). After incubation at 35 ± 2°C/24 h, plates counted within 25 to 250 colonies were used to calculate bacterial population numbers. The bacterial count was reported as a log of colony-forming unit (log CFU/g) for shrimp samples and (log CFU/mL) for water samples. Studies were repeated in duplicates, and the results were demonstrated as means ± SD.

Colonies of different characteristics of shape, size, and color were selected randomly from plate count agar (PCA) and incubated on additional nutrient agar and trypticase soy agar (TSA, Lab M, UK) slants. Bacteria selected from PCA media were then cultured on DeMan Rogosa Sharpe agar (MRS Lab M, UK) and incubated at 30°C for 24 h. Bacterial colonies on MRS media suspected as lactic acid bacteria (LAB) were Gram-stained and identified biochemically with catalase and oxidase tests [35]. In parallel, commercial API 20E strips (BioMérieux, France) were also used to identify randomly selected bacteria from PCA [36].

2.6.3. Vibrio Count. Shrimps (5 g) were transferred into a sterile bag with 45 mL of alkaline peptone water (lab M, UK) containing 1% NaCl. Samples were homogenized for 2 min using a stomacher (Seward Stomacher 400 circulator, UK). Previous steps were repeated with flour, molasses, and feed samples. Similarly, water samples (1 mL) were vortexed in 9 mL alkaline peptone water (APW, pH 8.6) (lab M, UK) containing 1% NaCl, and all samples were incubated at 37°C for 24 hrs.

Plating of an enrichment culture of thiosulphate-citrate-bile salts sucrose (TCBS) green and yellow colonies on TCBS plates counted as *Vibrio*-like colonies. Random colonies were transferred to trypticase soy agar (TSA) slants (Lab M, UK) containing 1% NaCl. After incubation at 37°C for 24 h, the isolates were subjected to biochemical tests such as oxidase reaction and API 20E diagnostic strips [37, 38].

2.7. Statistical Analysis. Statistical analysis was performed using IBM SPSS Statistics 25 software (IBM Corporation, NY, USA). The effects of treatment on growth performance, feed utilization, survival rate, and proximate composition of flocs and shrimps were analyzed using an independent sample *t*-test. Water quality parameters were compared by two-way repeated-measure ANOVA, with treatment as the main factor and sampling date as the repeated measures factor. Bacterial counts were compared using an independent sample *t*-test (IBM SPSS Statistics version 25). Results were expressed as the mean ± SE. Mean differences were compared by Duncan's multiple range test. A probability value (p) of less than 0.05 was used to indicate statistically significant differences.

3. Results

3.1. Water Quality. A significantly lower dissolved oxygen concentration (5.35 ± 0.067), higher turbidity (64.27 ± 2.36), and higher biofloc volume (18.40 ± 0.53) were found in the molasses treatment compared to the wheat flour treatment (Table 2). Mean values of pH were not significantly different. Slightly lower NH_3 (0.03 ± 0.002) and NO_2 (0.353 ± 0.029) concentrations were recorded in molasses treatment with no significant difference from the wheat flour treatment.

3.2. Growth Performance and Survival Rate. Results revealed significantly higher final weight (12.37 ± 0.04), weight gain (12.34 ± 0.04), average daily weight gain (0.096 ± 0.0003), weight gain per week (0.68 ± 0.002), and specific growth rate (4.70 ± 0.002) in the molasses treatment as shown in Table 3. On the other hand, the survival rate was significantly higher in the wheat flour treatment (99 ± 0.29) as

Table 2: Water quality parameters (mean ± SE) on biofloc systems for *Litopenaeus vannamei* using different carbon sources within a 128-day culture period.

Water quality parameter	Carbon sources		Significance
	Wheat flour	Molasses	
DO (mg/L)	5.59 ± 0.062	5.35 ± 0.067	0.014
NH3 (mg/L)	0.0307 ± 0.001	0.03 ± 0.002	0.834
NO2 (mg/L)	0.363 ± 0.017	0.353 ± 0.029	0.789
pH	7.05 ± 0.070	7.15 ± 0.058	0.253
Turbidity (NTU)	56.87 ± 2.55	64.27 ± 2.36	0.042
Floc volume (mL/L)	16.27 ± 0.589	18.40 ± 0.532	0.012

Notes. DO: dissolved oxygen; NH3: ammonia; NO2: nitrite.

compared with molasses (93.7 ± 0.17). Total biomass per tank (71.16 ± 0.42 kg) and the biomass increase percentage (395.33 ± 2.32) were both significantly higher in the wheat flour treatment than molasses (69.5 ± 0.23 kg and 386.11 ± 1.26, respectively).

3.3. Feed Utilization. Results of the current study revealed significantly lower feed conversion ratio (1.37 ± 0.01) and higher protein efficiency ratio (1.92 ± 0.015) were observed in the wheat flour treatment as compared with the molasses treatment (Table 4).

3.4. Proximate Composition of Bioflocs. The proximate composition of the biofloc was different in the two treatments, indicating that biofloc chemical composition is affected by different carbon sources (Table 5). Crude protein and fat content were slightly higher in wheat flour treatment (19.90 ± 0.81%) and (2.05 ± 0.084%), respectively, than those recorded in molasses treatment (18.64 ± 0.63%) and (2.04 ± 0.04%), respectively, with no significant differences. Ash content in the bioflocs from the molasses treatment (22.25 ± 0.46) was significantly higher ($p < 0.05$) than in the wheat flour treatment (14.32 ± 0.36). Bioflocs that were derived from the tanks provided with wheat flour showed significantly higher fiber content (16.08 ± 0.30) than molasses flocs (12.7 ± 0.27%).

3.5. Biochemical Composition of Shrimp. Results of the present study revealed that crude protein (73.52 ± 0.35), lipids (3.91 ± 0.17), and fiber (7.22 ± 0.36%) were all higher in shrimp bodies from wheat flour treatment compared to molasses, with significant differences in protein and lipids only (Table 6).

3.6. Bacterial Community

3.6.1. The Bacterial Community of Water. Total heterotrophic bacterial count (THB) in water samples in BFT supplemented with sugarcane molasses and flour were estimated as 3.4×10^5 CFU/mL and 1.2×10^5 CFU/mL, respectively, with no significant difference ($p > 0.05$) (Table 7). Selected colonies on MRS media from water samples of both treatments were catalase-negative and Gram-positive; thus, they were identified as lactic acid bacteria (LAB) according to Kaktcham et al. [39].

Each type of carbohydrate supplementation had different effects on the total *Vibrio*-like bacterial count (TVC) in water. Wheat flour addition had a significant ($p < 0.05$) reduction to TVC (1.3×10^3 CFU/mL) compared to sugarcane molasses (1.4×10^4 CFU/mL) (Table 7). Consequently, the proportion of *Vibrio*-like count to total heterotrophic bacterial count (V/T) was significantly ($p < 0.05$) lower in the biofloc group supplemented with flour (0.01) than sugarcane molasses (0.04). In addition, no pathogenic *Vibrio* species were identified in this study. *Enterobacter cloacae* bacteria were identified from water samples in both BFT systems supplemented with flour and molasses (Table 8).

3.6.2. Bacteria Associated with Shrimp. The total bacterial count was recorded as $4 \times 10^5 \pm 0.06$ CFU/g in BFT supplemented with molasses, while it was recorded as $1.1 \times 10^5 \pm 0.28$ in BFT supplemented with flour with no significant difference ($p > 0.05$) (Table 9). *Vibrio*-like bacterial count from shrimp samples of BFT with molasses (1.32×10^5 CFU/g) was high compared to BFT with flour (1.9×10^4 logs CFU/g), $p < 0.05$. Accordingly, the proportion of *Vibrio*-like count to total heterotrophic bacterial count (V/T) was significantly ($p < 0.01$) lower in the biofloc group supplemented with flour (0.17) than sugarcane molasses (0.33). Pathogenic *Vibrio* spp. were not identified with shrimp samples. Bacteria identified associated with shrimp include *Cronobacter* spp. in BFT with molasses and *Enterobacter amnigenus* in BFT with flour using API 20E diagnostic strips (Table 8).

3.6.3. Bacteria Associated with Flour, Molasses, and Feed. Total bacterial count and *Vibrio*-like bacterial count were estimated as less than 1 log CFU/g in flour, molasses, and feed samples.

4. Discussion

4.1. Water Quality. All water quality parameters in both treatments were found to be within suitable ranges for *L. vannamei* culture as reported by Schneider et al. [40]. Many studies reported that biofloc technology with adding carbon sources leads to increased growth of microbial population and enhanced water quality [41–43].

Our results were in agreement with Panigrahi et al. [19] and Rajkumar et al. [16] in finding the treatment with molasses which had significantly lower DO and higher TSS, floc volume, and turbidity than that of wheat flour. The decreased dissolved oxygen concentration in molasses treatment may be due to the increase in oxygen consumption by microbial activity. As molasses treatment supported higher microbial community represented by higher turbidity and higher floc volume, the higher solubility of molasses than the complex carbon source (wheat flour) provided higher turbidity and higher floc volume [15]. Insignificant differences in pH records were inconsistent with the results obtained by Rajkumar et al. [16], while Khanjani et al. [15] observed a significantly higher pH level in molasses treatment than the wheat flour treatment. These findings comply

Table 3: Growth performance parameters (mean ± SE) of *Litopenaeus vannamei* in treatments with two different carbon sources after a 128-day culture period.

Growth performance	Carbon sources		Significance
	Wheat flour	Molasses	
Final weight (g)	11.98 ± 0.05	12.37 ± 0.045	0.001
Weight gain (g)	11.95 ± 0.05	12.34 ± 0.045	0.001
ADWG (g/day)	0.094 ± 0.0004	0.096 ± 0.00036	0.001
G/W	0.65 ± 0.0027	0.68 ± 0.0025	0.001
SGR%	4.68 ± 0.003	4.70 ± 0.0029	0.001
Biomass (kg)	71.16 ± 0.42	69.50 ± 0.23	0.025
Biomass increase percentage	395.337 ± 2.32	386.11 ± 1.26	0.025
Survival rate%	99 ± 0.29	93.7 ± .17	0.001

Notes. ADWG: average daily weight gain; G/W: weight gain per week; SGR%: specific growth rate%.

Table 4: Feed utilization parameters (mean ± SE) of *Litopenaeus vannamei* in treatments with two different carbon sources after a 128-day culture period under a biofloc system.

Parameter	Carbon sources		Significance
	Wheat flour	Molasses	
FCR	1.37 ± 0.01	1.48 ± 0.021	0.009
PER	1.92 ± 0.015	1.77 ± 0.027	0.009

Notes. FCR: feed conversion ratio; PER: protein efficiency ratio.

with Panigrahi et al. [19], who found that TAN and NO_2 concentrations were lower in molasses treatment than in wheat flour treatment with no significant difference in TAN concentration between the two treatments. The higher degradation of molasses might lead to a higher number of heterotrophic bacteria utilizing ammonia and nitrite, which improves water quality [44]. Generally, soluble carbon sources such as molasses dissolved rapidly in water and release the carbon needed for the microorganism's bioactivity, resulting in the direct elimination of nitrogen compounds [17, 18, 45].

4.2. Growth Performance and Survival Rate. The high growth performance and survival rate obtained with the two biofloc treatments can attribute to the appropriate water quality. Particularly, ammonia and nitrite are considered primary limiting factors in shrimp survival [46].

Our study indicated higher SGR, ABW, and ADG in the molasses treatment than in the wheat flour treatment as reported by Panigrahi et al. [19] and Khanjani et al. [15]. The lower survival rate in molasses treatment may explain the higher individual growth performance parameters. In contrast, a higher survival rate was found in the wheat flour treatment compared to the molasses treatment. Wheat flour treatment showed a better survival rate and total yield which is consistent with the result found by Rajkumar et al. [16].

4.3. Feed Utilization. High bacterial and zooplankton densities, high nutritional values, and the improved water quality in this study resulted in better feed utilization efficiency of *L. vannamei*. These results agreed with Rajkumar et al. [16] who found that the usage of wheat flour as a carbon source resulted in a significantly lower FCR and higher PER compared to molasses addition. Moreover, Tinh et al. [47] found that molasses addition resulted in a higher FCR compared to starch treatment. Complex carbohydrates (bran, flour, and starch) have a slow effect as they should be degraded before being utilized by microorganisms. Therefore, their effects on regulating water quality were sluggish [15]. However, insoluble carbohydrates could be consumed directly by shrimps as food supplements and improve their growth performance [7, 45].

4.4. Proximate Composition of Bioflocs. Proximate chemical compositions of the bioflocs from this study have an appropriately high level of crude protein and crude lipid for omnivorous *L. vannamei* which is inconsistent with Rajkumar et al. [16], Maicá et al. [48], Khanjani et al. [15], and Tinh et al. [47]. The different protein content in bioflocs could be resulting from other environmental conditions such as temperature, total suspended solid concentration, salinity, light intensity, phytoplankton, stocking density, zooplankton, and bacterial communities [49].

4.5. Biochemical Composition of Shrimp. The significantly higher protein, lipid, and lower ash content found in shrimp with wheat flour treatment, as opposed to the molasses treatment, agreed with Rajkumar et al. [16] and Khanjani et al. [15]. The higher protein, lipid, and fiber content of biofloc in wheat flour treatment resulted in a higher protein, fat, and fiber content in the shrimp body. This agreed with Xu and Pan [50] who confirmed that the presence of good proximate composition in the biofloc resulted in better growth of shrimp.

4.6. Bacterial Community

4.6.1. The Bacterial Community of Water. In this study, the total heterotrophic bacteria (THB) count in water samples in BFT supplemented with sugarcane molasses and flour was dominant with no significant difference ($p > 0.05$). Panigrahi et al. [19] reported that THB count in water

Table 5: Proximate composition of bioflocs (Mean ± SE) produced in biofloc systems with the addition of different carbon sources.

Biofloc analysis	Carbon sources		Significance
	Wheat flour	Molasses	
Protein	19.904 ± 0.81457	18.649 ± 0.63502	0.235
Ether extract	2.0567 ± 0.08407	2.0413 ± 0.04664	0.874
Ash	14.32 ± 0.36478	22.2533 ± 0.46760	0.001
Crude fiber	16.0867 ± 0.30548	12.7 ± 0.27551	0.001
Nitrogen free extract	47.6327 ± 0.82431	44.3560 ± 0.87684	0.011

Table 6: Proximate composition of shrimp body (Mean ± SE) after a 128-day culture period in biofloc systems with the addition of two different carbon sources.

Constituent	Carbon sources		Signifecanceday
	Wheat flour	Molasses	
Protein	73.52 ± 0.359	72.41 ± 0.184	0.012
Ether extract	3.91 ± 0.172	3.19 ± 0.0984	0.001
Ash	13.20 ± 0.192	14.93 ± 0.208	0.001
Crude fiber	7.22 ± 0.36135	6.995 ± 0.283	0.622
Nitrogen free extract	2.15 ± 0.45560	2.47 ± 0.308	0.562

Table 7: Heterotrophic and *Vibrio*-like bacterial count (CFU/mL) in water samples from biofloc systems supplemented with sugarcane molasses and wheat flour.

	Sugarcane molasses	Wheat flour	Significance
Heterotrophic bacterial count*	$3.4 \times 10^5 \pm 0.08$	$1.2 \times 10^5 \pm 0.30$	0.082
Vibrio-like bacterial count	$1.4 \times 10^4 \pm 0.26$	$1.3 \times 10^3 \pm 0.10$	0.002
V/T	0.04	0.01	0.001

Notes. Asterisks indicate bacterial count were represented as means ± SD. V/T: *Vibrio* count/total heterotrophic bacterial count.

improved for Pacific white shrimp (*L. vannamei*) under a biofloc system with multigrain flour (11.54×10^6 CFU/mL) and molasses (10.1×10^6 CFU/mL).

Lactic acid bacteria (LAB) were isolated and identified as associated with both supplements in this study. It had an important role in the control of fish pathogens, thanks to the production of lactic acid, other organic acids, and bacteriocin inhibitory substances [39, 51]. This might explain the absence of pathogenic *Vibrio* spp. Several studies have reported high resistance of shrimps to pathogenic *Vibrio* in BFT through probiotic administration such as LAB [52, 53]. Flour addition had a significant ($p < 0.05$) reduction in total *Vibrio*-like bacterial count (TVC) in water compared to molasses, which is in agreement with Kumar et al. [54] and Panigrahi et al. [19].

Enterobacter Cloacae were identified from water samples in both BFT systems. *Enterobacter* spp. are natural commensals of shrimp microbiota [55]. It might exist in water samples associated with both molasses and flour supplements due to the accumulation of shrimp commensals, as biofloc is considered a zero-exchange closed water system. *Enterobacter cloacae* have a potential application in aquaculture water treatment. Shu et al. [56] reported *E. cloacae* isolation from aquaculture water and showed significant heterotrophic denitrification ability. The efficiencies of inorganic nitrogen removal by *E. cloacae* were 72.27 to 96.44%. *Enterobacter cloacae* were described as probiotic additives that improve weight gain and prevent and control fish diseases such as yersiniosis [57]. On the other hand, water was not permitted for any human consumption, as public health considerations were reported for *E. cloacae* as opportunistic bacteria causing pneumonia, urinary tract infections, wound infections, and bacteremia [58, 59]).

4.6.2. Bacteria Associated with Shrimp. Water quality reflects on shrimp bacterial quality, as the total bacteria count of shrimps was in correspondence with the count estimated in water. The total number did not exceed the maximum level of the Egyptian Organization for Standardization and Quality Control [60] of shrimp (less than 10^6 CFU/g). On the other hand, the *Vibrio*-like bacterial count from shrimp samples of BFT with molasses was higher than that with flour. Accordingly, the proportion of *Vibrio*-like count to total heterotrophic bacterial count (V/T) was significantly ($p < 0.01$) lower in the biofloc group supplemented with flour. Flour had been documented as an effective

Table 8: Biochemical test results of different isolated bacteria using API 20E diagnostic strips from water and shrimp samples in biofloc systems supplemented with sugarcane molasses and wheat flour.

		Sugarcane molasses		Wheat flour	
		Water	Shrimp	Water	Shrimp
		Enterobacter cloacae	*Cronobacter* spp.	*Enterobacter cloacae*	*Enterobacter amnigenus*
ONPG		+	+	+	+
Arginine dihydrolase		+	+	+	+
Lysine decarboxylase		—	—	—	—
Ornithine decarboxylase		+	+	+	+
Citrate		+	+	+	+
H_2S		—	—	—	—
Urease		—	—	—	—
TDA		—	—	—	—
Indole		—	—	—	—
Voges-Proskauer		+	+	—	+
Gelatinase		—	—	+	—
	Glucose	+	+	+	+
	Mannitol	+	+	+	+
	Inositol	—	—	—	—
	Sorbitol	+	+	+	+
Acid from:	Rhamnose	+	+	+	+
	Sucrose	+	+	+	—
	Melibiose	+	+	+	+
	Amylose	+	+	+	+
	Arabinose	+	+	+	+

Notes. ONPG: O-nitrophenyl-β-D-galactpyranoside, β-galactosidase test; H_2S: hydrogen sulfide test; TDA: tryptophan deaminase reaction.

Table 9: Total bacterial count and *Vibrio*-like bacterial count (CFU/g) in shrimp samples from biofloc systems supplemented with sugarcane molasses and wheat flour.

	Sugarcane molasses	Flour	Significance
Total bacterial count*	$4 \times 10^5 \pm 0.06$	$1.1 \times 10^5 \pm 0.28$	0.07
Vibrio-like bacterial count	$1.32 \times 10^5 \pm 0.14$	$1.9 \times 10^4 \pm 0.27$	0.018
V/T	0.33	0.17	0.006

Notes. Asterisks indicate bacterial count were represented as means value ± SD. V/T: *Vibrio* count/total heterotrophic bacterial count.

prophylactic method against *Vibrio parahaemolyticus* infection that decreased shrimp mortality in the biofloc system. Shrimp fed with flour (*Dunaliella* sp.) provided in the diet daily for 15 days showed 93% survival compared to glucan (87%) and infected control (79%) survivals [61].

Other bacteria associated with shrimp include *Cronobacter* spp. in BFT with molasses and *Enterobacter amnigenus* in BFT with flour. *Enterobacter* sp. is a natural habitat of shrimp gastrointestinal tract [55]. The clinical behavior of *E. amnigenus* is similar to that of *E. cloacae*, a taxonomically related species [62]. *E. cloacae* was isolated from water, which might reflect the existence of the closely related *E. amnigenus* in shrimp. *E. amnigenus* was reported as the major food spoilage bacteria [63]. However, the *Cronobacter* group of pathogens is associated with diseases especially in neonatal and elderly [64]. Certain species of *Cronobacter* such as *sakazakii* are considered opportunistic food-borne pathogens [65], which may reflect on food safety in BFT systems supplemented with sugarcane molasses.

The reported Molasses contain some vitamins and minerals; it contains iron up to 5% of [66]. Iron is essential for all living organisms, and human pathogenic bacteria require ferrous ions for their growth and virulence [67]. The expression of virulence factors in *Vibrio anguillarum* was recorded by Lages et al. [68] regulated by iron levels and temperature. Several animal models were used to demonstrate increased susceptibility to bacterial infections after injection of iron-containing compounds. Iron has been shown to increase the lethality and morbidity of *Neisseria meningitides*, *Listeria monocytogenes*, and *V. vulnificus* infections [69–71]. That is why cautious and regulated use of molasses as a source of carbon should be taken into consideration.

4.6.3. Bacteria Associated with Flour, Molasses, and Feed. Low-moisture foods (LMF with water activity, Aw < 0.85) including wheat flour and shrimp feed did not support most microbial growth [72]. Generally, an Aw of 0.95 or higher is required to support microbial growth [73]. Flour water activity (Aw) level ranged from 0.3 to 0.6 ± 0.02 at 25°C [74]. *Vibrio*'s best generation time was recorded as 16.4 min corresponding to water activity (a(w) of 0.992 ± 0.005) [75]. Molasses samples are characterized by an anaerobic condition which is not considered a favorable condition for heterotrophic bacteria and *Vibrio* growth [73]. This might explain the absence of total bacterial count and *Vibrio*-like bacterial count in flour, molasses, and feed samples. Therefore, flour, molasses, or feed did not interfere with the total bacterial count and *Vibrio*-like count results in this study.

5. Conclusion

This study highlights the importance of different carbon sources in improving the biofloc system, shrimp performance, and quality. The results guarantee the use of wheat flour over molasses to enhance growth performance, survival rate, feed utilization, bioflocs composition, and shrimp body composition with a better bacterial community.

Data Availability

The research data associated with a paper is available, and the data can be accessed.

Conflicts of Interest

The authors declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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