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Effects of carbohydrate sources on a biofloc nursery system for whiteleg shrimp (*Litopenaeus vannamei*)

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ABSTRACT

In biofloc technology carbohydrate is added to stimulate the biofloc growth, the latter helps to improve water quality, reduce the need for water exchange and may serve as natural shrimp feed. The large diversity among possible carbohydrate sources makes the selection of a suitable carbohydrate difficult. This study investigated how corn starch addition compared to molasses addition affected water quality, biofloc and periphyton proximate composition, shrimp production parameters, diurnal fluctuations and distribution of carbon and nitrogen in whiteleg shrimp (*Litopenaeus vannamei*) culture system. The results showed that both corn starch and molasses addition treatments resulted in low ammonium nitrogen levels in the water. The total suspended solids and volatile suspended solids in both treatments increased over time and were not significantly different among treatments. The protein content in the dry matter of the biofloc varied from 34% to 48%, being higher in the molasses treatment. The same was observed for the protein content in the dry matter of the periphyton which ranged between 16% and 26%. The corn starch treatment resulted in significantly higher shrimp growth rate, production, average body weight, and lower FCR compared to molasses addition. Water quality was stable on a daily basis, but changed over the weeks. Carbon and nitrogen accumulations in the system were not significantly different among treatments.

1. Introduction

The role of aquaculture in fish supply for human consumption has been increasing since the 1980s (FAO, 2018). To meet the food fish demand of a growing global population, global aquaculture production is expected to increase by 62% from 2010 to 2030, especially in countries like China, India, and in Southeast Asia and Latin America (World Bank, 2013). The future expansion of the aquaculture industry should preferably occur in a resource efficient way (Crab et al., 2012; World Bank, 2013). This includes better use of basic natural resources such as water and land, and fish feed which are the major pre-requisites for aquaculture activities.

The biofloc technology may provide the necessary solutions. The addition of organic carbohydrate (CHO) in the biofloc system provides an energy source for microbial organisms to immobilize ammonia or nitrate into microbial biomass (Avnimelech, 1999). This process helps lowering ammonia and nitrite levels, thereby reducing the need for water exchange (Gao et al., 2012; Hu et al., 2014). At the same time, the generated microbial biomass forms aggregates, called biofloc, which serves as natural food for culture species and increases feed use efficiency (Burford et al., 2004; Emerenciano et al., 2012). The application of biofloc systems is also beneficial for immunological responses of whiteleg shrimp against infectious agents (Ekasari et al., 2014; Kim et al., 2014; Verma et al., 2016).

The diversity of possible CHO sources causes difficulties in the adoption of the biofloc system. Potential CHO sources may include simple ones such as molasses, glycerol, and glucose, and complex ones such as flour and starch (Crab et al., 2010; Guo et al., 2006; Nikodinovic-Runic et al., 2013). Different CHO sources yield different nutritional values of the biofloc (Crab et al., 2010; Rajkumar et al., 2016; Wei et al., 2016). In addition, they have varying effects on the composition of the microbial community in the biofloc (Deng et al., 2018; Wei et al., 2016), the production (Khanjani et al., 2016; Rajkumar et al., 2016) and the immunity of the cultured fish and shrimp (Ekasari et al., 2014; Verma et al., 2016). The underlying mechanism for some of these differences may lie in the complexity of CHO structure (Khanjani et al., 2016; Wei et al., 2016). These results altogether indicate the importance of selecting a suitable CHO for a successful biofloc technology.

In biofloc technology, feed and CHO represent major sources of

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organic material entering the system. Following feeding, the oxygen consumption and ammonia excretion by fish significantly increase, creating high fluctuations in ammonia concentration on a daily basis (Zakęś et al., 2006). However, following the addition of CHO in tilapia culture the ammonia, nitrite, and nitrate concentrations did not show significant diurnal fluctutations (Hu et al., 2014). Equivalent information regarding diurnal changes of water quality parameters in the biofloc system for whiteleg shrimp remains scarce. Besides, it is currently unknown how the carbon and nitrogen inputs are accumulated in different compartments (e.g. shrimp, biofloc, water) of the biofloc culture of whiteleg shrimp. Further research on nutrient recycling in biofloc system is still needed.

This research aimed at comparing the effects of different CHO on water quality, biofloc and periphyton proximate composition, shrimp production parameters, the diurnal fluctuation of water quality parameters following the addition of feed and CHO, and quantifying the accumulation of nutrients (input carbon, and nitrogen) in different compartments of the biofloc shrimp culture. Corn starch and molasses were used as representatives of complex dietary CHO and simple by-product CHO, respectively.

2. Materials and methods

The experiment was performed at the animal research facility of Wageningen University (Carus), the Netherlands from January to February of 2018.

2.1. Pre-treatment

Six weeks prior to the experiment, biofloc was produced in 6 indoor plastic tanks (900-L total volume; 600-L working volume) stocked with 1.5 kg/m^3 of 93 g individual weight tilapia. The fish were fed twice per day with a 33% protein feed at 2.5% body weight per day, assuming a feed conversion ratio (FCR) of 1.2. Per kilogram of feed fed, 1.52 kg corn starch was added to the tank following each feeding to maintain a C:N ratio of 20 for optimal biofloc growth (Pérez-Fuentes et al., 2016).

The tanks were provided with continuous aeration, and 12 h/12 h dark/light regime. The salinity of culture water was increased gradually, starting at 0 ppt and reaching 20 ppt by the end of the pre-treatment period. Before pumping water to treatment tanks, tilapia from pre-treatment tank were removed, and pre-treated water was mixed together and adjusted to the salinity of 25 ppt using fine salt (Suprasel® Classic, Akzo Nobel Functional Chemicals B.V., the Netherlands).

2.2. Experimental design

The experiment was conducted with corn starch and molasses as treatments of CHO source, each in triplicate. The same 6 tanks as in the pre-treatment period were used. The tanks were placed indoor with temperature control, 12 h/12 h dark/light regime providing the average light intensity of 9800 lx. Prior to stocking shrimp, treatment tanks were filled with 4 cm of living sand bottom, 600 L of flocculated water. Culture water was continuously aerated by installation of an air ring (made from PVC tube with holes) at the bottom of the tank.

2.3. Experimental animal and feeding

Shrimp (0.075 ± 0.006 g per individual) were obtained from CreveTec, Ternat, Belgium, and stocked at the density of 250 shrimp/m^2 into their respective treatment tanks. Shrimp were fed twice per day at 9 AM and 3 PM with 34% protein feed (CreveTec) at the feeding level of 8% BW per day, and assumed FCR of 0.7, reaching a maximum feeding rate of 43 g/m^3/day.

After feeding, corn starch and molasses were added immediately to their respective treatments. For each kilogram of shrimp feed fed, 0.6 kg of corn starch or 1.1 kg of molasses were added to maintain an input C:N ratio of 12. A summary of shrimp feed ingredients and proximate composition of shrimp feed and CHO sources can be found in Tables 1 and 2.

Table 1
Shrimp diet composition (CreveTec, Ternat, Belgium).

Ingredients	%
Fishmeal	16
Fish oil	1
Wheat gluten	10
Soybean meal	10
Krill protein hydrolysate	1
Wheat flour	27.6
Wheat	20
Wheat bran	10
Cholesterol	0.2
Soya lecithin	0.5
Monocalcium phosphate	1.6
CaCO3	0.4
Premix	1
Lysine HCL	0.3
DL-methionine	0.2
L-Threonine	0.2

Table 2
Nutritional composition of shrimp diet and carbohydrates on dry weight (DW) basis.

Proximate compositions	Values
Feed:	
Dry matter (g/kg wet weight)	911
Crude protein (g/kg)	341
Ash (g/kg)	69
Energy (kJ/g)	20
Corn starch:	
Dry matter (g/kg wet weight)	884
Crude protein (g/kg)	3
Ash (g/kg)	1
Energy (kJ/g)	18
Molasses:	
Dry matter (g/kg wet weight)	630
Crude protein (g/kg)	85
Ash (g/kg)	171
Energy (kJ/g)	18

2.4. Water quality monitoring

During the pre-treatment and experimental periods, water temperature and pH were monitored using electric probes, and ammonia concentration was checked daily and nitrite concentration irregularly using test kits (Merck MQuant®). Dissolved oxygen was monitored and maintained at the level above 6 mg/L during the entire duration of the experiment. There was no water exchange during the experimental period.

2.5. Sample collection and analysis

The culture system was divided into 5 compartments, including shrimp, water (filtrate after filtration at 1.5 µm pore size), biofloc (materials remaining on 1.5 µm pore size filter), periphyton (materials sticking on the tank wall), and sediment. Samples were taken periodically to assess the effects of treatments on and the distribution of nutrients in each compartment, as detailed below.

Shrimp samples were collected as a composite sample at the beginning and separately from each tank at the end of the experiment for determination of average body weight and survival rate. Samples were freeze-dried for one week, and grinded prior to the analysis of dry matter and ash (following ISO 6496, 1999), crude protein (following

Table 3
Shrimp production parameters at the end of 5-week experiment.

Treatments	Body weight (g/ind)	Biomass (g)	Weight gain (g/ind)	Survival (%)	FCR	FCR (incl. CHO)	Growth rate (% BW/day)
Corn starch	2.5 ± 0.1	355 ± 15	2.4 ± 0.1	96 ± 1	1.3 ± 0.1	2.0 ± 0.1	10.1 ± 0.3
Molasses	1.3 ± 0.0	178 ± 4	1.2 ± 0.1	90 ± 2	2.6 ± 0.1	5.5 ± 0.1	8.1 ± 0.1
P values	***	***	***	*	***	***	**

Presented values are the averages ± SD of each treatment, with * $P < .05$, ** $P < .01$, *** $P < .001$.
FCR – feed conversion ratio.
FCR (incl. CHO) – feed conversion ratio taking into account the amount of carbohydrate added.

ISO 5983, 2005), energy (following ISO 9831, 1998), total carbon (TC) and total nitrogen (TN) contents (using Dumas analyzer). Samples of feed, corn starch, and molasses were also preserved for the analysis of similar parameters as with shrimp samples. In addition, mineral contents in corn starch and molasses including phosphorus (P), calcium (Ca), iron (Fe), magnesium (Mg), potassium (K), and manganese (Mn) were analyzed using the segmented flow analyzer (SAN + +, Skalar Analytical B.V., the Netherlands). Corn starch and molasses were separately dissolved in stetile de-ionized water to a similar concentration, and used for a 5-day biological oxygen demand (BOD_5) assay following the standard protocol (APHA, 1995).

Water samples were collected at the beginning of the experiment and weekly onwards. Unfiltered water samples were analyzed for total suspended solids (TSS), volatile suspended solids (VSS), chlorophyll *a*, b, and c following the Standard Methods for the Examination of Water and Wastewater (APHA, 1995). Settleable solids using Imhoff cone were not determined because TSS gives a more accurate estimation of biofloc biomass (Xu et al., 2016). The filtrates, after having gone through a glass microfiber filter of 1.5 µm pore size, were acidified with 3 N HCl to pH of 2–3, and analyzed for total carbon (TC), inorganic carbon (IC), total nitrogen (TN), total ammonia nitrogen (TAN), nitrite and nitrate (NO_x), phosphate phosphorus (PO_4-P) using a segmented flow analyzer (SAN + +, Skalar Analytical B.V., the Netherlands).

Biofloc samples were collected at the beginning of the experiment and the end of weeks 1, 3, and 5 by filtration through glass microfiber filter of 1.5 µm pore size. Samples were kept at -20 °C until further analysis of proximate composition (following APHA, 1995), total energy following ISO 9831 (1998), total carbon and total nitrogen contents (using LECO CN 628 Dumas analyzer, LECO Instrumente GmbH., Germany).

Soil samples were collected at the start of the experiment, and the end of weeks 3 and 5. Samples were kept at -20 °C until further analysis of proximate composition, total carbon and total nitrogen contents employing the same protocols as with shrimp samples.

Periphyton samples were collected at the end of weeks 3 and 5, as periphyton was not present at the beginning of the experiment. To allow easy periphyton collection, two white plastic solid sheets of 15 cm in width were attached to the basin wall, going all the way from the tank bottom to above the water surface. Periphyton samples were preserved at -20 °C prior to the determination of their proximate composition, energy, carbon and nitrogen contents.

2.6. 24 h measurements

To examine the diurnal fluctuation of water quality parameters, on day 32 of the experiment, water and biofloc samples were taken one hour after the first feeding (9 AM) and every 3 h onwards for a period of 24 h. Water samples were analyzed for TAN, NO_X, TSS, VSS, total carbon and total nitrogen; biofloc samples were analyzed for total carbon and total nitrogen, using the aforementioned methods.

2.7. Data analysis

Statistical analysis was performed using IBM SPSS Statistics 25 software (IBM Corporation, NY, USA). The effects of treatment on shrimp production and proximate composition, and nutrient accumulation were analyzed using One-way ANOVA. The effects of treatment on weekly and diurnal water quality, biofloc-related and periphyton-related parameters were analyzed using Repeated measure ANOVA. A probability value (P) of less than 0.05 was used to indicate significant differences.

3. Results

3.1. Effects on shrimp production

A summary of shrimp production parameters is shown in Table 3. After the 5-week culture period, shrimp body weight in the corn starch treatment (2.47 ± 0.13 g/ind) was significantly higher than that in the molasses treatment (1.32 ± 0.01 g/ind) ($P < .05$). Shrimp survival rate was above 90% in both treatments, and significantly higher in the corn starch treatment ($P < .05$). Total shrimp biomass in the corn starch treatment was double of that in the molasses treatment. Specific growth rate was significantly higher in the corn starch treatment ($P < .05$). Overall, the corn starch addition resulted in better production parameters compared to the molasses addition.

Proximate analysis showed that on average, final shrimp samples had higher C:N ratio, dry matter and energy contents, but lower ash content compared to the initial shrimp sample (Table 4). Shrimp in the corn starch treatment contained more dry matter and total energy, and a higher C:N ratio, compared to shrimp in the molasses treatment ($P < .05$). Meanwhile, shrimp in the corn starch treatment contained significantly less ash than in the molasses treatment ($P < .05$). Crude protein content in shrimp was not different among treatments ($P > .05$).

3.2. Effects on water quality

Overall, the total ammonia nitrogen levels remained low throughout the experimental period. The TAN concentration was highest at the beginning of the experiment (Fig. 1). The TAN concentration decreased from 0.38 mg/L to less than 0.1 mg/L in both treatments during the first week and remained below that level subsequently. Measured TAN concentrations in the molasses treatment in

Table 4
Proximate composition of initial and final shrimp samples.

Parameters	Initial sample	Final samples		
		Corn starch	Molasses	*P* values
Dry matter (g/kg WW)	130	217 ± 8	199 ± 4	**0.033**
Ash (g/kg DW)	188	148 ± 4	156 ± 3	**0.045**
Crude protein (g/kg DW)	741	728 ± 12	742 ± 10	0.212
Total energy (kJ/g DW)	18.9	20.3 ± 0.1	19.8 ± 0.0	**0.001**
Carbon-nitrogen ratio	3.5	3.8 ± 0.1	3.6 ± 0.1	**0.044**

Values are means (± SD). Initial sample was analyzed on a batch sample of post-larvae ($n = 1$). Probability (P) values given only relate to final samples. P values ($P < .05$) in **bold** indicate significant effects.

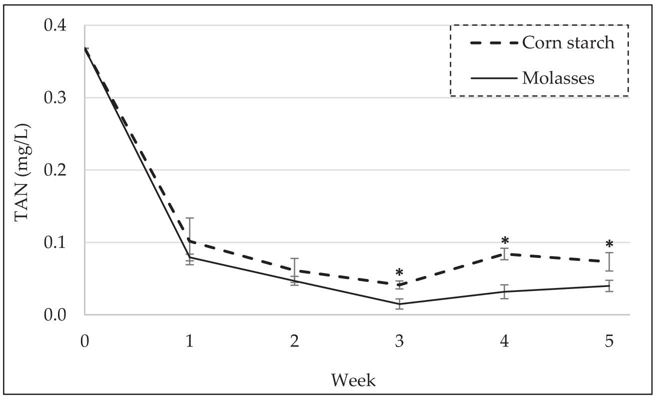

Fig. 1. Weekly changes of ammonium nitrogen (TAN) concentration in water by treatment (carbohydrate source). Values are means (± SD) of three replicate tanks per sampling time in each treatment. The asterisks (*) indicate weeks with significant difference among treatments ($P < .05$).

weeks 3, 4, and 5 were significantly lower than those in the corn starch treatment ($P < .05$).

In both treatments, the NO_x-N concentration increased during the first week of the experiment (Fig. 2). The NO_x-N concentration declined from weeks 1 to 4, and increased in the last week of the experiment. The differences in the NO_x-N concentration were not significant between the two treatments ($P > .05$). When verified, the NO_2-N concentration was always below 0.5 mg/L.

The fluctuation of dissolved nitrogen showed relatively similar patterns among treatments (Fig. 3). Organic and inorganic nitrogen increased in the first week of the experiment, and then decreased form week 1 to week 4. In week 5, organic and inorganic nitrogen content in water showed a slight increase in both treatments. The inorganic nitrogen in water was not different among treatments at all sampling points ($P > .05$), while the organic nitrogen in the molasses treatment was significantly higher than that in corn starch treatment from week 2 onwards ($P < .05$).

The dissolved inorganic carbon remained relatively constant throughout the experimental period (Fig. 3), but was significantly higher in the molasses treatment than in the corn starch treatment in weeks 4 and 5 ($P < .05$). Meanwhile, the organic carbon (OC) in water of the molasses treatment kept increasing and was higher than that of the corn starch treatment in weeks 2, 3, 4, and 5 ($P < .05$). Chlorophyll a concentration was not different among treatments, showing an increase between weeks 1 and 3, and a decrease between weeks 3 and 5 (Fig. 4).

Repeated measures ANOVA showed that all measured parameters

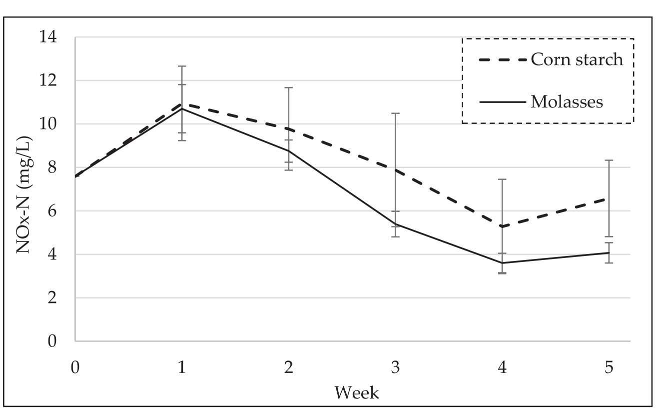

Fig. 2. Weekly changes of nitrite and nitrate nitrogen (NO_x−N) concentration in water by treatment (carbohydrate source). Values are means (± SD) of three replicate tanks per sampling time in each treatment.

changed significantly over weeks ($P < .05$) (Table 5). The effects of treatments on TC, IC, OC, ON, TAN in water were significantly different from each other ($P < .05$). However, their effects on TN, IN, NO_x−N, PO_4-P, and chlorophyll parameters were not different ($P > .05$).

3.3. *Effects on biofloc production*

The biofloc growth was evaluated based on changes in TSS and VSS concentrations in the water. At all sampling points, TSS concentration was not different between the two treatments ($P > .05$) (Table 6). TSS concentration decreased from the beginning to the first week of the experiment and increased from the end of week 1 onwards (Fig. 5). Changes in VSS concentration showed similar pattern to that of TSS (Fig. 5) and were not significantly different among the two treatments ($P > .05$).

The average protein content in biofloc in both treatments was higher than 40% of the dry matter (Table 6). Biofloc protein content significantly changed during the culture period ($P_{Time} < 0.05$). Molasses yielded a biofloc with significantly higher protein content ($P < .05$) than in the corn treatment. Ash and total energy contents, and the C:N ratio of biofloc did not change significantly in time and were not significantly different among treatments ($P > .05$).

3.4. *Effects on periphyton production*

Periphyton was removed from all tank walls at the beginning of the experiment to ensure similar starting conditions. At the end of week 3, the periphyton biomass was not different among treatments ($P > .05$), but was significantly higher in the molasses treatment at the end of week 5 ($P < .05$) (Fig. 6). Repeated measures ANOVA showed that total periphyton biomass increased over time and was significantly higher in the molasses treatment ($P < .05$) (Table 7). Periphyton in the molasses treatment had significantly higher protein content, and significantly lower C:N ratio compared to those of the corn starch treatment ($P < .05$). The ash and organic matter contents in periphyton were comparable among treatments ($P > .05$).

3.5. *Diurnal fluctuations of water quality and biofloc*

3.5.1. *Water quality parameters*

Fig. 7 showed that within 24 h period, carbon and nitrogen contents in water were relatively stable. The total dissolved nitrogen in water was comparable among treatments, while the total dissolved carbon in water in the corn starch treatment was lower than that in the molasses treatment on the sampling day (Table 8) ($P > .05$). This was consistent with the results of the weekly measurements shown in Fig. 3. However, the repeated measure analysis showed that TC, OC, TN, ON, TAN, and PO_4-P changed significantly during the day ($P < .05$).

3.5.2. *Biofloc*

Biofloc volume in terms of suspended solids was measured at the beginning and end of the 24 h measurement. In both treatments, there was a relatively small increase in TSS after 24 h, however, the difference between biofloc volume at the start and end of the 24 h period was insignificant ($P > .05$). Biofloc carbon and nitrogen contents were not significantly different among different sampling times of the day in both treatments ($P > .05$), averaging 339 g carbon and 57 g nitrogen per kilogram of biofloc. The average C:N ratio of biofloc in both treatments was 5.9 ± 0.3., and constant during the day ($P > .05$).

3.6. *Nutrient accumulation*

During the experiment, the absolute amount of carbon and nitrogen in the system increased in both treatments (Fig. 8). One-way ANOVA showed that the percentages of both retained carbon and nitrogen were not significantly different among treatments ($P > .05$). Carbon

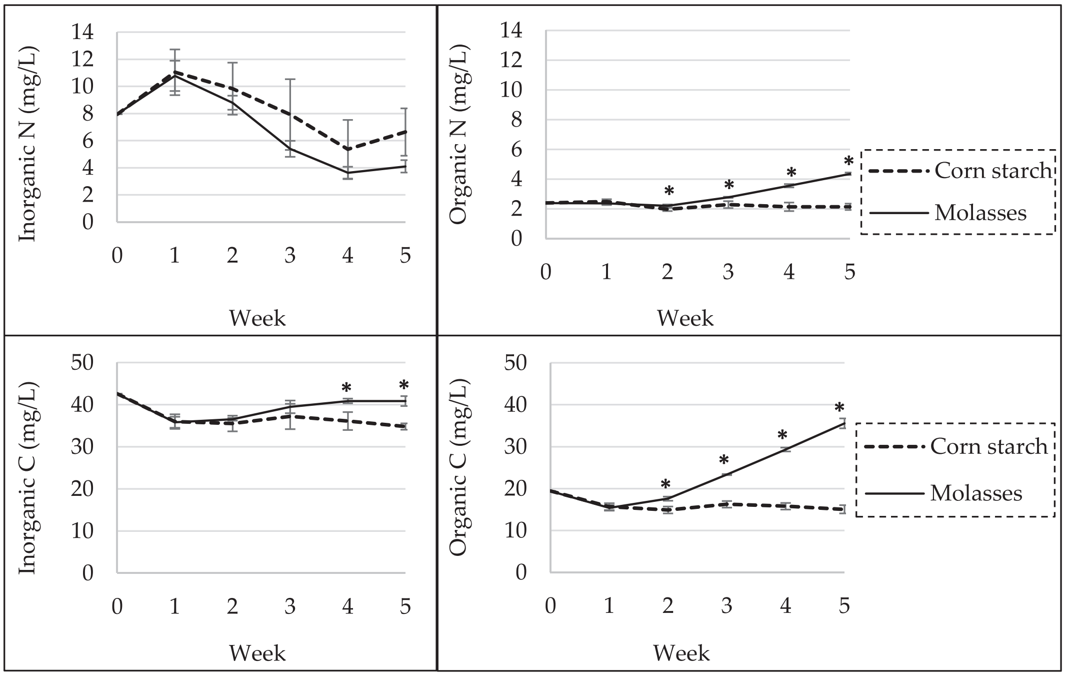

Fig. 3. Weekly changes of dissolved nitrogen (N) and carbon (C) in water by treatment (carbohydrate source). Values are means (± SD) of three replicate tanks per sampling time in each treatment. The asterisks (*) indicate weeks with significant difference among treatments (P < .05).

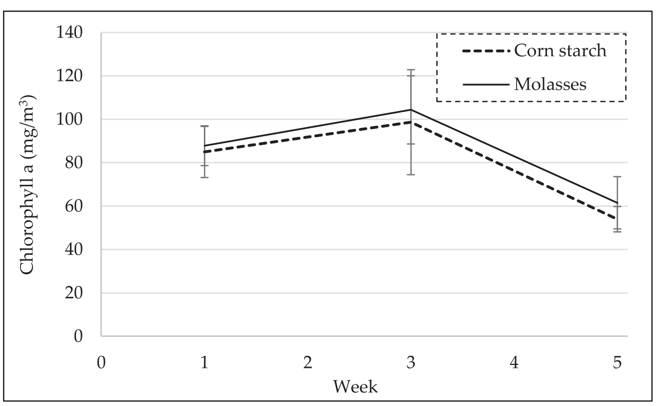

Fig. 4. Changes of chlorophyll *a* concentration in water by treatment (carbohydrate source). Values are means (± SD) of three replicate tanks per sampling time in each treatment.

Table 5

Summary of selected parameters in water compartment by treatment (carbohydrate source). Values are means (± SD) of six sampling times for each treatment. Probability (P) values in bold indicate significant effects (P < .05).

Parameters	Treatments		P values		
	Corn starch	Molasses	Treatment	Time	Interaction
TC (mg/L)	52 ± 2	63 ± 10	**0.000**	**0.000**	**0.000**
IC (mg/L)	36 ± 2	39 ± 2	**0.036**	**0.006**	**0.005**
OC (mg/L)	16 ± 2	24 ± 7	**0.000**	**0.000**	**0.000**
TN (mg/L)	10 ± 3	10 ± 2	0.510	**0.000**	0.457
IN (mg/L)	8 ± 3	7 ± 3	0.205	**0.000**	0.168
ON (mg/L)	2.2 ± 0.3	3.1 ± 0.8	**0.000**	**0.000**	**0.000**
TAN (mg/L)	0.07 ± 0.03	0.04 ± 0.02	**0.005**	**0.000**	0.196
NO_x-N (mg/L)	8 ± 3	7 ± 3	0.211	**0.000**	0.177
PO_4-P (mg/L)	1.1 ± 0.3	1.0 ± 0.2	0.192	**0.000**	0.817
Chl a (mg/m^3)	79 ± 24	85 ± 22	0.608	**0.000**	0.920
Chl b (mg/m^3)	61 ± 21	70 ± 24	0.094	**0.002**	0.940
Chl c (mg/m^3)	95 ± 30	111 ± 39	0.115	**0.002**	0.984

Table 6

Biofloc biomass in terms of total suspended solids (TSS) and volatile suspended solids (VSS), and biofloc proximate composition by treatment (carbohydrate source). Values are means (± SD) of six sampling times for each treatment. Probability (P) values in bold indicate significant effects (*P* < .05).

Parameters	Treatments		P values		
	Corn starch	Molasses	Treatment	Time	Interaction
TSS (mg/L)	419 ± 99	431 ± 134	0.709	**0.000**	**0.000**
VSS (mg/L)	298 ± 79	314 ± 113	0.542	**0.000**	**0.006**
Ash (g/kg DW)	292 ± 56	281 ± 60	0.678	0.083	0.715
Crude protein (g/kg DW)	394 ± 28	426 ± 53	**0.001**	**0.000**	0.097
Total energy (kJ/g DW)	10 ± 3	13 ± 7	0.077	**0.000**	**0.013**
Carbon:nitrogen ratio	6.5 ± 1	6.8 ± 1.1	0.579	0.397	0.473

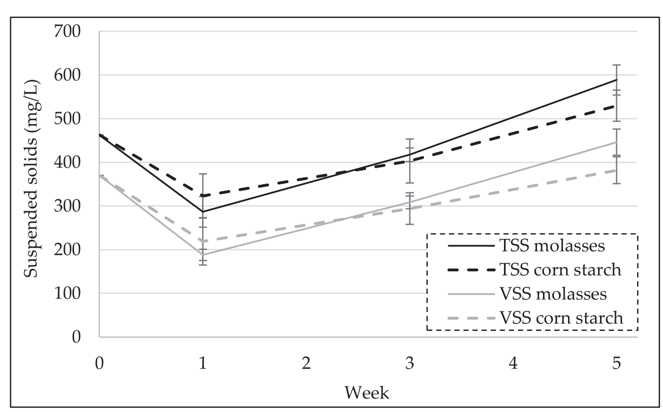

Fig. 5. Changes of total suspended solids (TSS) and volatile suspended solids (VSS) in water of corn starch and molasses treatments. Values are means (± SD) of three replicate tanks per sampling time in each treatment.

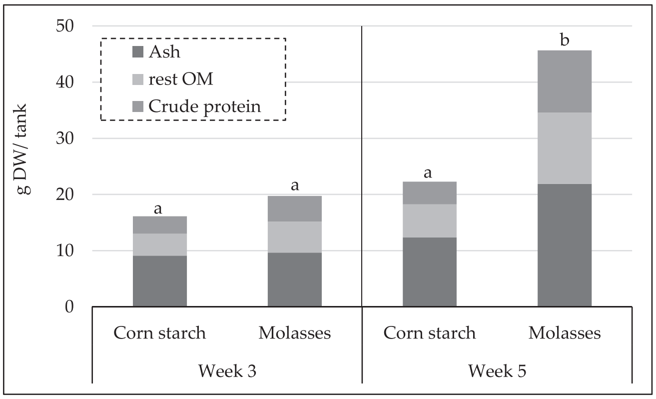

Fig. 6. Absolute periphyton biomass at the end of weeks 3 and 5 by treatment (carbohydrate source). Values are means of three replicate tanks per sampling time for each treatment. For each week, different letters on the top of columns indicate significant differences (P < .05).

Table 7
Proximate composition of periphyton by treatment (carbohydrate source). Values are means (± SD) of two sampling times for each treatment. Probability (P) values in bold indicate significant effects (P < .05).

Parameters	Treatments		P values		
	Corn starch	Molasses	Treatment	Time	Interaction
Total biomass (g DW/tank)	19 ± 8	33 ± 15	**0.032**	**0.013**	0.057
Ash (g/kg DW)	550 ± 39	487 ± 43	0.121	0.302	0.668
Crude protein (g/kg DW)	192 ± 23	237 ± 18	**0.042**	0.813	0.495
Organic matter (g/kg DW)	450 ± 39	513 ± 43	0.121	0.302	0.668
Carbon:nitrogen ratio	6.4 ± 0.1	6.2 ± 0.1	**0.043**	0.112	0.764

retention efficiency in corn starch and molasses treatments were 15 and 17% of total carbon input, respectively. The majority of the retained carbon in the corn starch treatment accumulated in the shrimp and in the sediment. In the molasses treatment, retained carbon was more equally distributed in all compartments of the culture system.

Regarding nitrogen, 43 and 28% of the input were retained in corn starch and molasses treatments, respectively. In the corn starch treatment, retained nitrogen mainly accumulated in the shrimp. Meanwhile, accumulated nitrogen in the molasses treatment was more equally distributed among shrimp, biofloc and periphyton.

Table 8
Summary of selected parameters from 24 h water measurement by treatment (carbohydrate source). Values are means (± SD) of nine sampling times for each treatment. Measurement was done on day 32, at 3-h intervals. Probability (P) values in bold indicate significant effects (*P* < .05).

Parameters	Treatments		P values		
	Corn starch	Molasses	Treatment	Time	Interaction
TC (mg/L)	52 ± 1	77 ± 2	**0.000**	**0.011**	0.391
IC (mg/L)	37 ± 2	42 ± 1	**0.007**	0.074	0.653
OC (mg/L)	15 ± 1	35 ± 1	**0.000**	**0.001**	**0.013**
TN (mg/L)	8.1 ± 1.7	7.7 ± 0.4	0.737	**0.000**	0.108
IN (mg/L)	5.9 ± 1.7	3.5 ± 0.4	0.132	0.088	0.513
ON (mg/L)	2.2 ± 0.3	4.2 ± 0.3	**0.000**	**0.000**	**0.003**
TAN (mg/L)	0.1 ± 0.03	0.1 ± 0.04	0.150	**0.000**	**0.000**
NO_x-N (mg/L)	5.8 ± 1.8	3.5 ± 0.4	0.132	0.165	0.529
PO_4-P (mg/L)	1.0 ± 0.29	0.9 ± 0.04	0.687	**0.000**	0.920
TSS (mg/L)	505 ± 34	554 ± 51	0.211	0.065	0.391

3.7. Mineral content and BOD5 of corn starch and molasses

Molasses contained higher concentrations of all analyzed minerals, especially potassium, compared to corn starch (Table 9). In total, corn starch and molasses treatments received 260 and 484 g/tank, respectively, for the whole experimental duration. Consequently, the molasses treatment had greater absolute mineral inputs and concentrations in water compared to corn starch treatment. Regarding the 5-day biological oxygen demand (BOD_5), each milligram dry matter of molasses consumed 0.1 ± 0.02 mg oxygen, while this value for corn starch was 0.2 ± 0.04 mg oxygen (P < .05).

4. Discussion

4.1. Shrimp

The survival percentage (90–96%) and specific growth rate (8–10% BW/day) of shrimp in this research were high and comparable to previous studies on white leg shrimp nursery (Arias-Moscoso et al., 2018; Correia et al., 2014; Khanjani et al., 2016; Serra et al., 2015). In research covering a 3–4 month whole culture cycle, lower survival

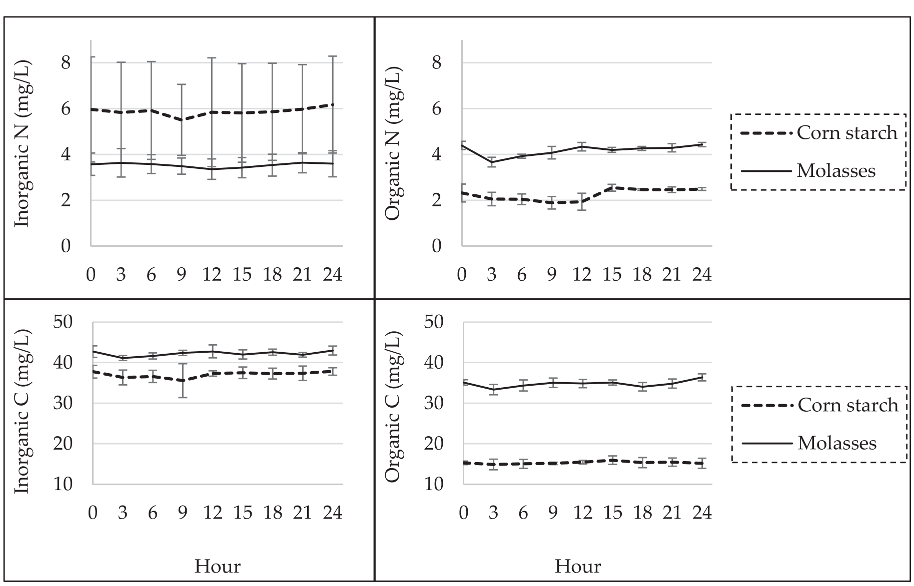

Fig. 7. Diurnal fluctuation of dissolved nitrogen (N) and carbon (C) in water by treatment (carbohydrate source). Values are mean (± SD) of three replicate tanks per sampling time in each treatment. Measurement was done on day 32, at 3-h intervals. Shrimp feeding was done one hour prior to and five hours after the first sampling.

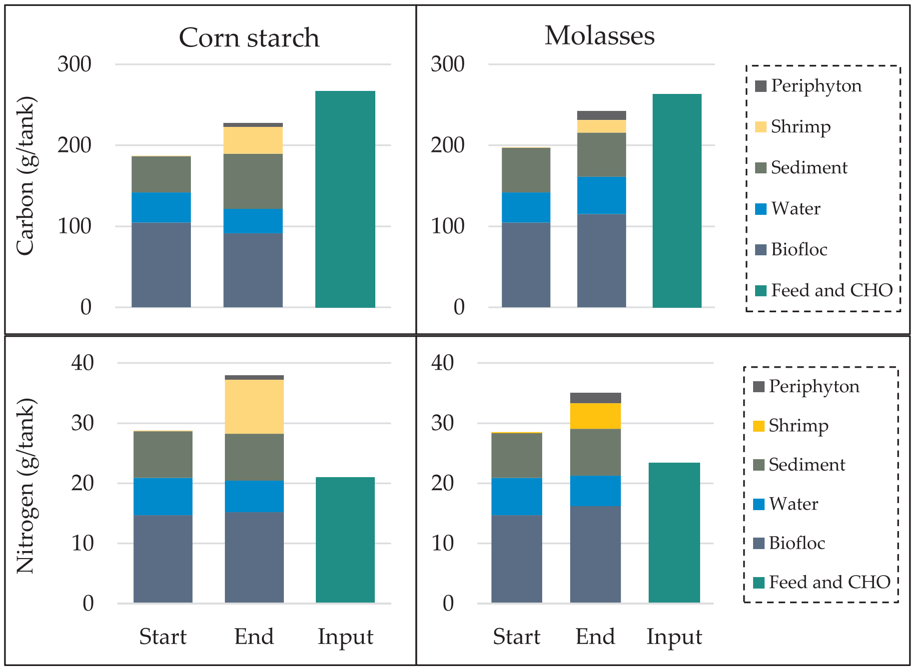

Fig. 8. Carbon and nitrogen distributions in different compartments of the culture system, and total nutrient input from feed and carbohydrates. Values are means of three replicate tanks by treatment (carbohydrate source).

Table 9
Mineral content, absolute input, and concentration in water of carbohydrate sources.

	P	Ca	Fe	Mg	K	Mn
Mineral content of carbohydrates (g/kg):						
Corn starch	0.13	0.08	0.001	0.02	0.06	0
Molasses	0.24	0.56	0.080	0.30	34.2	0.03
Molasses/corn starch	1.9	6.7	80	12	561	–
Absolute input from carbohydrates (g):						
Corn starch	0.03	0.02	0	0.01	0.02	0
Molasses	0.12	0.27	0.04	0.140	16.5	0.02
Molasses/corn starch	4.0	14	–	14	825	–
Concentration of minerals provided from carbohydrates in water (mg/L):						
Corn starch	0.05	0.03	0	0.02	0.03	0
Molasses	0.20	0.45	0.07	0.23	27.5	0.03

percentages of shrimp (82–93%) were reported with an overall specific growth rate of 7.6% BW/day (Khoa et al., 2020; Krummenauer et al., 2016).

In this research, a C:N ratio of 12 was used for both treatments. This ratio was of intermediate level, and recommended for biofloc culture of whiteleg shrimp (Panigrahi et al., 2019; Xu et al., 2018, 2016). Nevertheless, the addition of corn starch yielded significantly better shrimp production. This production enhancement was thought to be due to the higher stability of environmental conditions in the corn starch treatment, in particular the dissolved organic carbon and nitrogen, compared to that in the molasses treatment (Fig. 3). Stable water quality reduces stress and improves growth and survival of cultured shrimp (Janeo et al., 2009). Lower and more stable organic carbon and nitrogen concentrations in the water of corn starch treatment suggests that the microbial community in this system differed in composition and was better in functionality than in the molasses treatment (Jiang et al., 2020; Moss and Pruder, 1995). However, the conclusion that molasses should be replaced with corn starch in biofloc technology was not yet drawn, as one may argue that corn starch is a dietary ingredient for both human and animal, and of greater value compared to molasses, which is a by-product from sugar industry (Guo et al., 2006; Nikodinovic-Runic et al., 2013).

The two carbohydrate sources used in this research differed significantly in mineral content, especially in potassium. Previous research also reported that iron, potassium and manganese concentrations in molasses were approximately 17, 50, and 70 times, respectively, higher than in starches (Heuzé et al., 2016, 2015; Prasanthi et al., 2017). Besides, the phosphate concentration in the molasses treatment was higher than in the corn starch treatment and previously reported values in conventional shrimp pond (Casillas-Hernández et al., 2007). However, whether these differences contributed to the difference in shrimp growth among treatments is unclear. Overall, beside maintaining a suitable C:N ratio, the choice of CHO source is of prime importance in biofloc technology, as CHOs have different effects on biofloc nutritional content (Crab et al., 2010; Rajkumar et al., 2016; Wei et al., 2016), microbial diversity (Deng et al., 2018; Wei et al., 2016) and production of culture animal (Rajkumar et al., 2016). The latter was also found in the present study.

4.2. Water

Between the two CHOs, molasses contained mostly simple sugars, and was expected to be more easily utilized by microbial organisms, resulting in more rapid improvement in water quality (Heuzé et al., 2015; Wei et al., 2016). This effect was observed in weeks 3, 4, and 5 when significantly lowered TAN levels were obtained in the molasses treatment compared to the corn starch treatment. However, both treatments resulted in low TAN of 0.02–0.1 mg/L, which was sufficient for shrimp growth (Lin and Chen, 2001). However, the BOD_5 of corn starch was twice as high as the BOD_5 of molasses (0.2 and 0.1 mg O_2/mg DM, respectively), indicating differences in microbial activities. This may concur with a shift in microbial community composition, as different CHO sources have varied effects on biofloc microbial community (Deng et al., 2018). However, since both treatments in this research reacted similarly in term of water quality (e.g. TSS, VSS, TAN), it would be safe to assume that the switch of carbon source did not create imbalances. In the aerated biofloc systems used, the higher oxygen demand in the corn starch treatment did not cause problems.

In new systems, where there is no or low level of biofloc, biofloc concentration is often seen to increase continuously during the culture period (Xu et al., 2016; Xu and Pan, 2013). However, the TSS and VSS concentrations in this experiment decreased during the first week. This is due to the high biofloc concentration (> 460 mg/L) relative to the low amount of shrimp feed at the beginning of this experiment. Therefore, the nutrient input was too low to maintain the initial biofloc volume. Over the experimental period, total dissolved nitrogen decreased while TSS and periphyton biomass increased, suggesting that waste nitrogen was immobilized into microbial biomass.

Toward the end of the experiment, both dissolved organic carbon and nitrogen in the molasses treatment increased. However, the mechanisms of this occurrence could not be properly elucidated. One possibility is that while both treatments received the same amount of feed, shrimp growth in the molasses treatment was slower. Therefore, part of the feed in the molasses treatment was not eaten, and degraded releasing also organic carbon and nitrogen.

4.3. Diurnal fluctuation

The repeated measures analysis of 24 h water measurements showed that dissolved carbon and nitrogen fluctuated during the day ($P < .05$). In systems where feed and CHO are added twice per day, significant fluctuation of carbon and nitrogen contents following the addition of feed and CHO is expected. Nevertheless, Fig. 7 showed that the total carbon and nitrogen in water remained stable over 24 h. This may be due to the fact that these measurements were done toward the end of the experiment (day 32) when carbon and nitrogen input from carbohydrate (8.7 mg/L and 0.14 mg/L, respectively) were small compared to the amount present in the water at sampling time (65.5 mg/L for carbon and 8.13 mg/L for nitrogen). Although daily fluctuations were not significant over time, differences in organic and inorganic C and N concentrations were observed, as well as differences between corn starch and molasse treatments (Fig. 3).

4.4. Biofloc

Biofloc protein content was higher than 390 g/kg dry weight in both treatments. The average biofloc concentration reached 434 mg/L, presenting an extra source of nutrients for the cultured shrimp. The higher biofloc protein content observed in the molasses treatment likely stemmed from the fact that molasses contains more protein on dry weight basis (8.5%) than corn starch (0.3%), which was directly available to biofloc. This concurs with Kumar et al. (2017) also reporting that CHO with a high protein content resulted in biofloc with a high protein content. A regression of CHO protein content against protein content of biofloc from this study and Kumar et al. (2017) yielded a correlation coefficient of 0.91 ($R^2 = 0.83$). However, Kumar et al. (2017) used a feeding level of 1.5% body weight and maintained an assumed C:N ratio of 10, making combining the different treatments difficult. Therefore, it remains unclear how protein in CHO may change the protein concentration (on dry matter basis) in the biofloc.

We observed that as the biofloc concentration increased, its protein content increased, while its ash content decreased. This may be due to changes in the biofloc microbial composition. At high biofloc concentration, the bacteria part in biofloc became dominant over the algal content (Xu et al., 2016). In this research, algae in the system was outcompeted from week 3 onwards, showing a decrease in chlorophyll *a* concentration (Fig. 4) when biofloc concentration reached 403 mg/L in the corn starch and 417 mg/L in the molasses treatments (Fig. 5). A balanced biofloc system where neither algae nor bacteria is dominant is more beneficial for shrimp (Xu et al., 2016). Biofloc concentration of 400–600 mg/L is suitable for whiteleg shrimp culture (Schveitzer et al., 2013).

4.5. Periphyton

Periphyton production in this research reached 22–46 g DW/tank (Fig. 6) at the end of the experiment, with protein contents ranging from 19% to 24% (Table 7). Periphyton protein content, similarly to biofloc protein content, was significantly higher in molasses treatment. Studies on effects of carbohydrate type on periphyton were scarce. However, periphyton nutritional values were shown to be dependent on substrate type (Azim et al., 2005). Periphyton protein level in this research was comparable to the 25% found in whiteleg shrimp pond, and represented an extra source of nutrient for the culture animal (Kumar et al., 2017). Direct contribution of periphyton to intensive whiteleg shrimp culture has not been studied. However, it was shown that promoting periphyton growth by substrate addition increased whiteleg shrimp production in less intensive system (Kumar et al., 2017). In intensive system the aeration rate has a significant effect on evaporation, constantly reducing water level (Li et al., 2008). We observed that shrimp grazed on periphyton which mostly grew on tank wall at water-air interface, therefore, maintaining a constant culture water level may increase the availability of periphyton to the culture animal.

4.6. Nutrient accumulation

This research demonstrated that only 15–17% of carbon, and 28–43% of nitrogen input remained in the system at the end of the experiment. The nitrogen accumulated in shrimp in the corn starch treatment of this research is comparable to that in da Silva et al. (2013). However, the total 43% of the nitrogen input accumulated in the tank was slightly more than half of the 80% accumulation of the nitrogen input reported by (da Silva et al., 2013). The other 20% was assumed to be lost through denitrification and volatilization. In conventional culture ponds without CHO addition, 23% of carbon and 35% of nitrogen inputs (i.e. from feed and fertilizer) were assimilated in shrimp (Dien et al., 2018). This indicates that our system was less efficient in carbon use compared to the conventional system. Hu et al. (2014), utilizing a tilapia culture model, showed that the addition of CHO increased daily CO_2 emissions by 91%, however, it reduced the daily N_2O emissions by 83%, both of which are among major greenhouse gasses from aquaculture activities. Although this phenomenon has not been investigated in biofloc culture of shrimp, a similar trend can be expected to occur. Therefore, it can be controversial whether the adoption of the biofloc system should be encouraged, since this system was proven to be more efficient in water and nitrogen use, however, less efficient in organic carbon retention which may possibly have adverse effects on global warming (Gao et al., 2012; Hu et al., 2014). By employing mesocosms, the distribution of input nutrients among compartments in the system could be accounted. The unaccounted amount is considered lost through valorization. More insight in which factors contribute to a higher retention of nitrogen in a biofloc system merits further investigation.

5. Conclusions

The choice of organic carbon source plays an important role in the success of the biofloc system. Corn starch was superior to molasses for enhancing the growth of whiteleg shrimp. Once the biofloc is established, nitrogen waste can be efficiently controlled, resulting in relatively little diurnal fluctuation of nitrogen and carbon in culture water. However, the nutrient loss in biofloc systems, especially carbon loss is high, and ways to reduce C-loss from culture systems should be explored. Further research on improving nutrient use efficiency, either directly by culture animals or indirectly by trapping nutrients and making them available for other uses, is necessary.

Declaration of Competing Interest

The authors declare 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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