



Effect of molasses supplementation on growth performance, water quality and microbial dynamics during indoor rearing of pacific white shrimp *Penaeus vannamei* Boone, 1931

SANJOY DAS¹, DEBASIS DE¹, GOURANGA BISWAS², PREM KUMAR¹,
CHRISTINA LALRAMCHHANI¹, SUJEET KUMAR³ AND T. K. GHOSHAL¹

¹Kakdwip Research Centre of ICAR-Central Institute of Brackishwater Aquaculture, Kakdwip - 743 347, West Bengal, India

²Kolkata Regional Centre of ICAR-Central Institute of Fisheries Education, Kolkata - 700 091, West Bengal, India

³ICAR-Central Institute of Brackishwater Aquaculture, Chennai - 600 028, Tamil Nadu, India

e-mail: sanjoy125@yahoo.co.in; sanjoy.das1@icar.gov.in

ABSTRACT

A study was conducted with Pacific white-leg shrimp (*Penaeus vannamei* Boone, 1931) juveniles to ascertain the effect of molasses supplementation on the growth performance, culture environment and microbial dynamics of rearing water. The weight gain at the end of the experiment of 55 days duration were significantly ($p < 0.05$) higher in molasses supplemented group (12.08 ± 0.45 g) as compared to control (10.44 ± 0.34 g). Apparent feed conversion ratio (AFCR) was found significantly ($p > 0.05$) lower in case of molasses supplemented group with higher survival percentage. Supplementation of molasses also improved the rearing water quality. The total ammonia nitrogen (TAN) level was significantly ($p < 0.05$) lower in the rearing water of molasses supplemented tank (2.213 ± 0.165 ppm) compared to control (2.915 ± 0.191 ppm). There was no significant difference in total heterotrophic bacteria (THB) and total *Vibrio* (TV) load between the water of the two groups. However, the proportion of TV to THB was significantly lower in case of molasses supplemented group as compared to control. This study indicated that supplementation of molasses can significantly improve the growth performance of *P. vannamei* with better feed utilisation and also improves the water quality by reducing the level of toxic gas i.e. ammonia in water.

Keywords: Ammonia, C:N ratio, Molasses, *Vibrio*

Introduction

Shrimp farming is a fast growing sector of India as well as in the world and the frozen shrimp is considered as the most important export commodity among all Indian seafood. Due to the fast expansion of shrimp farming sector, the environmental issues and sustainability are of great concern (Naylor *et al.*, 2000). The shrimp farming in India is mainly export-oriented and the country earned foreign exchange equivalent to ₹3,25,203/- million through export of 0.59 million t of frozen shrimp during 2020-21 (MPEDA, 2021). Although this sector is highly productive and profitable, very often the sector has been ravaged by different devastating diseases like white spot disease, acute hepatopancreatic necrotic disease (AHPND) and taura syndrome (Thitamadee *et al.*, 2016). In shrimp aquaculture, some portion of the feed applied remains unconsumed and it accumulates to the pond bottom causing deterioration of water quality. External manipulation of Carbon:Nitrogen (C:N) ratio of pond water very often proved to be beneficial in shrimp farming in terms of improved feed conversion ratio and maintenance of good water quality parameters (Avnimelech, 2007; Kumar *et al.*,

2014). In the past, studies showed that for conversion of 1 g of total ammonia nitrogen (TAN), the requirement of carbon is around 10 g (Hari *et al.*, 2006). Various carbohydrate sources have been used by different workers for manipulation of C:N ratio in aquaculture with varying degrees of success (Hari *et al.*, 2006; Crab *et al.*, 2012). Molasses, rice bran, corn flour, tapioca and wheat bran are commonly used as carbon source in biofloc system (Wang *et al.*, 2015; Deng *et al.*, 2018). In addition to the beneficial effects on shrimp growth, immunity and feed conversion ratio (FCR); external carbohydrate supplementation also aids in reducing different nitrogenous metabolites including ammonia and nitrites, which are harmful to cultured shrimp (Avnimelech, 1999). In present days, the shrimp farmers show tendency to avoid water exchange in semi-intensive shrimp farming mostly due to unavailability of brackishwater with proper salinity level in the middle of culture period, requirement of high inputs in terms of labour and electricity/fuel during regular water exchange and also to avoid the danger of entry of pathogens during water intake. However, this water exchange can easily be avoided by systematic addition of external carbohydrates in the pond water throughout the shrimp culture period

(Kumar *et al.*, 2014). With this background, the present study was conducted to evaluate the growth performance and chemical as well as microbial water quality parameters during rearing of *P. vannamei* juveniles with external supplementation of carbon in the form of molasses.

Materials and methods

Experimental set up

The effect of extra carbohydrate supplementation in the form of molasses was evaluated on the growth of juvenile *P. vannamei*. The study was carried out for 55 days duration with four replicates each, in fibre-reinforced plastic (FRP) tanks (Inner measurement: L × B × H = 28" × 21" × 18") layered with clayey loam soil base with thickness of around 5 cm to simulate natural pond condition. The tanks were filled up to around 80 l of water (Salinity 11.5 ppt) from nearby brackishwater canal by filtering the water through nylon mesh (10 µm) and treated with commercial bleaching powder keeping the effective chlorine concentration around 20 ppm. The experiment was conducted at the research yard of Kakdwip Research Centre of ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Kakdwip, West Bengal, India (21°51'24.2"N, 88°11'3.7"E). Juvenile shrimps were procured from a commercial aquaculture farm located at Uttar Chandanpiri, South 24 Parganas, West Bengal, India (21°41'16.8"N, 88°17'38.4"E). Before stocking, the experimental shrimps were confirmed negative to white spot syndrome virus (WSSV) by two step PCR method as described by Kimura *et al.* (1996). Before initiation of the experiment, the shrimps were acclimatised for 10 days and fed with a commercial pellet diet with around 35% protein, two times a day. A total of 120 juveniles were randomly distributed to 8 FRP tanks at a density of 15 shrimps per tank. The initial body weight of individual shrimp was recorded during stocking. Four tanks were used as control tanks (A) and another four were designated as treatment tanks (B), where molasses was supplemented in the water. No water exchange was done throughout the experimental period. However to make up the evaporation loss, around 5 l of brackishwater (previously treated with commercial bleaching powder) with identical salinity was added to each experimental tank at 30 days of culture (DOC). Continuous aeration was provided with portable aerator fitted with aeration channel and diffusing stones.

Feed composition analysis

Proximate analysis of the feed was carried out following established methods (AOAC, 1995).

Feeding and supplementation of molasses

Commercial pellet feed for *P. vannamei* with 35% crude protein was used throughout the experiment. At

the beginning of the experiment, the feed was applied at the rate of 7.5% of the average body weight (ABW) and gradually reduced to 2.7%. Feed was applied twice (10:15 and 16:45 hrs) daily in two equally divided doses at the above-mentioned time schedule. In the treatment group, supplementation of extra carbon was done in the form of molasses containing 28% carbon and the amount of molasses supplementation was equal to the amount of feed as done by Kumar *et al.* (2014). Molasses was uniformly dispensed over tank water. The amount of organic carbon present in the used molasses was determined by the method of Walkley and Black (1934) as adopted by Kumar *et al.* (2014).

Water samples were collected in the morning between 10:00 and 11:00 hrs. Water temperature, salinity, dissolved oxygen (DO) and pH was measured daily for monitoring purpose. Water temperature was measured using a thermometer. Salinity, pH and dissolved oxygen were measured using a portable multimeter (Hach, USA, Model: HQ30D). Other water quality parameters including water alkalinity, TAN, nitrite-nitrogen (NO₂-N), nitrate-nitrogen (NO₃-N) and phosphate-phosphorus (PO₄-P) were estimated following the established methods (APHA, 1998) on 0, 20 and 55 days of the experiment.

Estimation of pH and organic matter of soil base

Soil samples from each tank was collected twice viz. just before stocking (DOC 0) and immediately after harvest (DOC 55). Samples were dried and estimation of pH and total organic matter was carried out as per method of Nelson and Sommers (1996).

Monitoring of microbial population

Water samples from the experimental tanks were collected for enumeration of total heterotrophic bacteria (THB) and total *Vibrio* (TV) at 0, 20 and 55 DOC. The enumeration of THB and TV was carried out from tank soil base twice i.e. at the beginning of the experiment (DOC 0) and on the last day of the experiment (DOC 55). Enumeration of both THB and TV was carried out as described by Kumar *et al.* (2014). After enumeration of THB and TV separately, ratio of TV/THB was also determined and all the microbial enumeration values were expressed as log₁₀ values.

Assessment of growth parameters of cultured shrimps

The body weight of individual shrimp was measured at the beginning (DOC 0) and end (DOC 55) of the experiment. The growth performance parameters such as apparent feed conversion ratio (AFCR), protein efficiency ratio (PER) and survival were estimated as per the method of Anand *et al.* (2013). Average daily weight gain and specific growth rate (SGR) were also calculated as follows:

Average daily weight gain (g day^{-1}) = (Average final weight - Average initial weight) / Culture period (days).

SGR (% per day) = $100 (\ln W_2 - \ln W_1) / \text{Duration of culture in days}$, where W_2 and W_1 are the average body weight of shrimp at the beginning and end of experiment, respectively (Wang *et al.*, 2003).

Statistical analysis

Independent samples t test ($p < 0.05$) was performed using statistical software SPSS 16.0 (IBM Corporation, USA) to ascertain whether there is any significant difference between two groups in terms of growth performance, water quality parameters, soil quality and microbial load. Before analysis, the data were checked for normal distribution using Shapiro-Wilk test of normality and followed the normal distribution.

Results and discussion

Composition of experimental feed

Moisture in the feed was estimated as 9.64%. Crude protein, crude lipid, crude fibre, total ash, acid insoluble ash and nitrogen free extract were estimated as 35.46, 4.7, 3.9, 14.48, 3.56 and 31.82% (dry matter basis), respectively. Total organic matter in feed was calculated as 85.52% (Table 1).

Water quality parameters of experimental tank water

At the beginning of the experiment during middle of October, the temperature ranged from 28.8 to 30.1°C. With progress of the experiment, the water temperature decreased at steady rate and at the end of experiment during first week of December, it ranged from 22.5 to 23.1°C. Water pH varied from 8.29 to 8.43 throughout the study period. DO ranged from 6.23 to 7.71 ppm during the experimental period. Water salinity was 11.5 ppt at the beginning of the experiment and it increased with progress

of the experiment. Salinity was in the range of 13.1 to 13.5 ppt at the end of experiment. There was no significant variation in TAN level in water between two groups at DOC 0 and 20. However at the end of the culture (DOC 55), the TAN level was significantly ($p < 0.05$) lower in the water of treatment tank (molasses supplemented tank) (2.213 ± 0.165 ppm) (B) compared to control (2.915 ± 0.191 ppm) (A). No significant differences were observed in $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ levels between molasses supplemented and control tanks. At the end of the culture period, a significantly higher ($p < 0.05$) $\text{PO}_4\text{-P}$ level was recorded in case of treatment tank (0.098 ± 0.008 ppm) as compared to control (0.061 ± 0.009 ppm). Alkalinity was found to be significantly lower ($p < 0.05$) in case of treatment tank as compared to control at DOCs 20 and 55. Significantly lower level of DO was observed in case of molasses supplemented tank at DOC 20, but no significant variation was observed at the end of the culture (DOC 55) (Table 2). Data relating to monitoring of temperature and salinity are not shown in the Table.

Soil quality parameters of experimental tank base

The results of soil quality parameters are illustrated in Table 3. There was no significant difference of soil pH between treatment and control tanks. However, the total organic matter in the soil base was significantly higher ($p < 0.05$) in case of molasses supplemented tank (2.13 ± 0.02 %) compared to control (1.84 ± 0.03 %) at the end of the experiment.

Microbial analysis

There was no significant difference in THB and TV load in the water samples of molasses supplemented and control tanks. However, when the relative proportion of TV in comparison to THB was measured, it was observed that TV/THB ratio was significantly ($p < 0.05$) lower in case of molasses supplemented tank compared to control group (Table 2).

No significant difference was observed in the level of TV between two groups in bottom soil base, whereas, significantly higher ($p < 0.05$) THB load was observed in the tank soil base of molasses supplemented tanks compared to control after 55 days of culture (Table 3).

Assessment of growth performance

The initial average body weight of the experimental shrimps in A and B was 2.22 ± 0.35 and 2.18 ± 0.05 g, respectively. The body weight gain at the end of culture period was significantly (independent t test, $p < 0.05$) higher in treatment (12.08 ± 0.45 g) as compared to control (10.44 ± 0.34 g). The survival percentages in control (A) and treatment (B) were 91.67 and 95%, respectively. Apparent feed conversion ratio (AFCR) was significantly

Table 1. Proximate composition of the feed

Nutrient	Proximate composition (%)
Crude protein	35.46
Crude lipid	4.7
Crude fibre	3.9
Total ash	14.48
Acid insoluble ash	3.56
Organic matter#	85.52
Moisture	9.64
Nitrogen free extract##	41.46
Gross energy (k cal 100 g ⁻¹)###	428.92

#Organic matter = $100 - \% \text{Ash}$

##Nitrogen free extract = $100 - (\text{Crude protein} + \text{Crude lipid} + \text{Total ash} + \text{Crude fibre})$

###Gross energy = $(\text{Crude protein} \times 5.6) + (\text{Crude lipid} \times 9.44) + \text{Crude fibre} \times 4.1 + (\text{Nitrogen free extract} \times 4.1)$

Table 2. Water quality parameters of the experimental tanks of the two experimental groups (Mean±SE)

DOC	Alkalinity (ppm)		Nitrite-N (ppm)		Nitrate-N (ppm)	
	A	B	A	B	A	B
0	149.5±3.5	155.5±3.3	0.019±0.001	0.019±0.001	0.139±0.005	0.146±0.010
20	181.0±1.29 ^a	166.0±1.8 ^b	0.596±0.003	0.611±0.031	0.403±0.005	0.379±0.015
55	186.5±1.7 ^a	177.5±2.2 ^b	0.650±0.025	0.595±0.060	0.375±0.020	0.359±0.021
DOC	TAN (ppm)		Phosphate-P (ppm)		pH	
	A	B	A	B	A	B
0	0.051±0.010	0.047±0.010	0.028±0.001	0.028±0.001	8.36±0.02	8.40±0.01
20	0.200±0.017	0.191±0.021	0.079±0.008	0.064±0.003	8.48±0.05	8.45±0.10
55	2.915±0.191 ^a	2.213±0.165 ^b	0.061±0.009 ^a	0.098±0.008 ^b	8.41±0.05	8.33±0.02
DOC	DO (ppm)		THB (log ₁₀ CFU ml ⁻¹)		TV (log ₁₀ CFU ml ⁻¹)	
	A	B	A	B	A	B
0	7.69±0.07	7.50±0.01	3.894±0.190	3.732±0.178	2.346±0.247	2.022±0.081
20	7.31±0.10 ^a	6.27±0.16 ^b	4.116±0.198	4.541±0.069	3.183±0.042	3.013±0.067
55	6.39±0.05	6.37±0.02	4.201±0.410	4.256±0.041	3.394±0.161	3.248±0.156
DOC	TV / THB ratio					
	A	B				
0	0.602±0.052	0.543±0.015				
20	0.778±0.034 ^a	0.664±0.018 ^b				
55	0.844±0.129 ^a	0.763±0.035 ^b				

p<0.05, pH and DO were checked on daily basis for monitoring purpose. Data of 0, 20 and 55 DOC are shown here

Table 3. Soil quality parameters of the experimental tanks of the two experimental groups (Mean±SE)

DOC	pH		Organic matter (%)			
	A	B	A	B		
0	8.73±0.02	8.73±0.02	0.90±0.01	0.92±0.01		
55	8.84±0.03	8.82±0.02	1.84±0.03 ^a	2.13±0.02 ^b		
DOC	THB (log ₁₀ CFU g ⁻¹)		Total <i>Vibrio</i> (log ₁₀ CFU g ⁻¹)		TV / THB ratio	
	A	B	A	B	A	B
0	6.055±0.370	6.123±0.307	3.274±0.031	3.298±0.022	0.541±0.009	0.543±0.027
55	6.562±0.900 ^a	7.756±0.263 ^b	5.308±0.172	5.312±0.205	0.824±0.072	0.680±0.046

p<0.05

(p<0.05) lower in case of molasses supplemented tank (1.49±0.05) over the control (1.72±0.06). The protein efficiency ratio (PER) was significantly (p<0.05) higher in case of molasses supplemented tank in comparison to control. Significant differences were also observed in case of average daily weight gain (g day⁻¹) and SGR. Both the growth parameters were improved with addition of molasses. The growth parameters are depicted in Table 4.

Manipulation of C:N ratio through supplementation of excess carbohydrate in the form of molasses was previously found to be beneficial in case of black tiger shrimp *Penaeus monodon* (Kumar *et al.*, 2014; Huang *et al.*, 2017). Although, the microbes present in aquaculture system are capable of utilising varying sources of carbon, molasses is more effectively utilised as it contains readily available sucrose, which is a disaccharide (Zhou

et al., 2002). The composition of experimental feed (Table 1) showed that the feed contained the essential nutrients at the required level (Li *et al.*, 2017). For shrimp culture, the most critical component of feed is the protein content. The crude protein content of the feed used in this study was 35.46%, which is considered to be the desirable range of protein requirement for *P. vannamei* (Lee and Lee, 2018). In the present study, it was observed that molasses supplementation significantly reduced the level of total ammonia nitrogen (TAN) in water at the end of culture. Kumar *et al.* (2014) also made similar observation in case of *P. monodon*. Although in microbial monitoring in tank water, no significant variation was observed in terms of THB and TV population between two groups, the relative proportion of TV with respect to THB was found significantly lower (p<0.05) in case of

Table 4. Growth parameters of shrimps in the two experimental groups (Mean±SE)

Parameters	A	B
	Mean±SE	(Mean±SE)
Initial average body weight (g)	2.22±0.35	2.18±0.05
Final average body weight (g)	12.66±0.32 ^a	14.27±0.46 ^b
Growth increment (g)	10.44±0.34 ^a	12.08±0.45 ^b
Feed conversion ratio (FCR)	1.72±0.06 ^a	1.49±0.05 ^b
Protein efficiency ratio (PER)	1.82±0.06 ^a	2.10±0.08 ^b
Average daily weight gain (g day ⁻¹)	0.190±0.006 ^a	0.220±0.008 ^b
Specific growth rate (SGR) (%)	3.162±0.064 ^a	3.410±0.056 ^b
*Survival (%)	91.67	95

*Mean value p<0.05

molasses treated group in comparison to control group at DOC 20 and 55. The presence of higher organic matter in the soil base of molasses supplemented tanks might be due to higher shrimp growth, higher microbial accumulation at bottom soil and presence of molasses. The decrease of water temperature during the progress of experiment was possibly due to onset of winter season. Although the alkalinity and dissolved oxygen levels were found slightly lower in case of molasses supplemented tank and both the parameters were within the desirable range (Chen, 1985). Higher phosphate-P level in molasses supplemented tank indicates that molasses supplementation can also be helpful for production of bloom in culture pond. Xu *et al.* (2016) also observed that with the supplementation of molasses, the level of nitrogenous gases including ammonia decreased in water and there was increase in phosphorus level. Among bacterial pathogens of shrimp, different species of *Vibrio* play significant role in occurrence of shrimp diseases leading to culture loss (Alavandi *et al.*, 2004; Austin and Zhang, 2006). The relative proportion of TV over THB could be an indication of health status of the cultured shrimp. In the present molasses-supplemented system, other heterotrophic bacteria dominated over *Vibrio*. In case of bottom soil of tank, higher load of THB indicates increased heterotrophs population in the system. Panigrahi *et al.* (2018) also reported similar type of findings. They observed that the dominance of *Vibrio* decreased in water with the increase of C:N ratio.

The growth of *P. vannamei* was significantly increased by 15.7% in molasses supplemented tank as compared to control (Table 4). The significantly lower feed conversion ratio in molasses supplemented tank over control indicates the better feed utilisation in case of experimental shrimp in molasses supplemented group. The study of Xu and Pan (2012) revealed that the addition of carbohydrates helped to form biofloc, which in turn improved digestive enzyme activity of shrimps. Significantly better protein efficiency ratio (PER), average daily weight gain and specific growth rate were also observed in case of experimental shrimp in molasses supplemented tank as compared to control

(Table 4). In addition to these, better survival was also observed in case of molasses supplemented tanks as compared to control. Similar type of observation was also made by Kumar *et al.* (2014) in case of *P. monodon*. The improved growth of shrimp with supplementation of additional carbohydrates was also reported by previous workers (Hari *et al.*, 2004; Wasielesky *et al.*, 2006).

The present study indicates that supplementation of molasses significantly improves the growth of *P. vannamei* with better FCR and it also significantly reduced the level of ammonia, which is toxic to the cultured shrimps. The survival of experimental shrimps was also found to be higher in case of molasses supplemented shrimp. Moreover, molasses supplementation also reduced the level of total *Vibrio* in proportion with total heterotrophic bacteria. Based on the findings of the present study, it can be ascertained that supplementation of molasses improves the growth performance of *P. vannamei* and also the culture environment leading to better health status of cultured shrimp. Thus at field level, the supplementation of molasses can be followed at certain interval for improving the quality of farmed *P. vannamei*. However, further studies are required on this aspect.

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