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Growth and digestive enzymatic activity of *Litopenaeus vannamei* raised in bio floc systems with different C/N ratios in ground saline water

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Abstract

The experiment was conducted to assess the effect of different C/N ratios in bio floc systems on growth performances and digestive enzymatic activity of *Litopenaeus vannamei*. The experiment was carried out using inland ground saline water at 15 ppt salinity fortified with potassium and magnesium ions. Bio floc developed with different C/N ratios as treatments T1 (5:1), T2 (10:1), T3 (15:1), T4 (20:1) and T5 (25:1) respectively by manipulating the C/N content in feed and water by adding rice bran as carbon source and without bio floc used as control. After optimum floc produced in experimental tanks the juveniles *Litopenaeus vannamei* (avg wt. 3.37±0.03 gm) were stocked. At the end of the experiment the weight gain % and SGR was found higher ($p < 0.05$) in treatments T3 (641.07c±12.55 and 3.29d±0.03) and T4 (650.00c±5.88 and 3.35d±0.01) respectively and in the treatment T1 and control group the growth rate was showed significantly similar ($p > 0.05$). A better FCR T4 (1.04a±0.01), T3 (1.12a±0.02) and T5 (1.16a±0.03) was observed significantly lower from control (2.23d±0.04) and T1 and T2. Similarly, significantly higher FER and PER was registered in T4 compared to control, T1 and T2 respectively. However, no significant difference in FER and PER was observed between T3 and T5. The 100% survival reported in treatments T1, T2, T3 and control groups with 99.33% and 94.33% survival reported in T4 and T5 groups. The different levels of C/N ratios in bio floc improved ($p < 0.05$) the digestive enzymes activities in hepatopancreas and intestine like amylase, lipase, cellulose and protease in the treatment groups compared with control. The present study elucidates the suitability of optimum C/N ratios in bio floc for enhancing the growth and digestive enzyme activities in *L. vannamei* in inland ground saline water. The finding could help in reducing the environmental concern saline waste water discharge from the shrimp pond to the land.

Keywords: Bio floc technology, C/N ratios, *L. vannamei*, environmental concern, inland ground saline water, minerals fortification

Introduction

The aquaculture is now recognized as the fastest emerging food production sector in Indian agriculture, aquaculture now converted into most traditional type farming to a remarkable enterprise by adopting latest technologies and techniques. In India the total potential suitable brackish water area of 1.20 million ha available for shrimp farming in which less than 16 % is utilised for culture practices. The area under culture was around 1.5-1.6 lakh ha during 1997-2007, and by 2008-09 it demolish to 1 lakh ha (22). In the increasing pressure on the land and water resources in the coastal areas for shrimp culture, a novel alternative will be to utilize the salt affected soils and saline aquifers in the inland regions of the country. Increasing inland salinity due to human activity has major economic, social and environmental concerns. Salinity in ground water and water logging by high irrigation affect the land productivity and also threaten the sustainability of agriculture. In India, nearly 8.62 million ha of land has been badly affected with the problem of soil salinity and 1.93 million KM² area is under laden with ground saline water, spreaded over the states of Rajasthan, Haryana, Punjab, Gujarat, Uttar Pradesh, Delhi, Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu. The non-utilization of ground saline water, the water table in these areas is rising with startling rate causing secondary salinization and water logging conditions. The ionic composition of saline groundwater generally reflects that of seawater, the ionic composition of saline groundwater differs with different geological areas. The difference in ionic composition of saline groundwater varies by natural according to salts composition of rocks and soil water interactions. Saline groundwater always having the deficiency of potassium, relative to equivalent salinity of seawater. Inland saline shrimp farming has several advantages to coastal shrimp farming.

Most arid and semi-arid regions where inland saline shrimp farming is carried out are several hundred kilometres away from the sea. The water pumped out from saline aquifers i.e. the saline ground water is almost sterile and there by chances of entry of a lethal virus through water source is zero. The inland shrimp farming also have several advantages over coastal shrimp farming due to threat of disease outbreaks and transmission is minimal. The saline groundwater in inland regions is recognised as a 'disease free environment' for shrimp farming (11).

Ground saline water deficient in potassium can be used for commercial shrimp farming through ionic manipulation by addition of muriate of potash (KCl) or sulfate of potash ($K_2SO_4 \cdot 2MgSO_4$) to the pond water. Addition of these fertilizers increase the K^+ ion level of pond water and make it more suitable for commercial shrimp farming. The better survival of shrimp post larvae reared in Inland saline water of low salinity fortified with potassium and magnesium (9,25). Shrimp farming in inland saline water is required the minimal discharge of saline water from the shrimp ponds. Bio-floc system is the only best alternatives for reducing the water exchange in inland shrimp farming. These practices reduced water exchange and also reduces the cost of production in shrimp farming. The bio floc technology is considered as rich microbial communities that form flocs in the water column. Bio floc technology is a technique of enhancing water quality through the addition of extra carbon to the aquaculture system. This accelerated nitrogen uptake by bacterial growth decreases the ammonium concentration more quickly than nitrification (12). Immobilization of ammonium by heterotrophic bacteria occurs much quicker because the growth rate and microbial biomass growth per unit substrate of heterotrophs are advanced than nitrifying bacteria (12). Several studies reported that shrimp raise successfully in systems that use limited or zero water exchange (20, 28). The bio floc is rich source of all nutrients and satisfy all the nutritional requirement of the rising animals but all the nutritional properties of the flocs are influenced by the type of carbon source used to produce the flocs (7). However, the purpose of the present study was to find the optimum C/N ratio for bio floc that is responsible for best shrimp growth and survival in inland ground saline water.

Materials and Methods

Experimental design and bio floc preparation

The experiment was conducted for 60 days at aquaculture wet lab located at Central Institute of Fisheries Education, Rohtak centre, Haryana, India. The Inland ground saline water with 15ppt salinity used for the experiments were pumped out from the farm tube well and kept it for settled in large cemented tanks for few days. The ionic composition of the water prior to experiment initiation were analysed and fortified with Potassium Chloride (KCl) and Magnesium Chloride ($MgCl_2$) (9). Potassium fortification was done using commercially available fertilizer Potassium Chloride (KCl) whose trade name is Muriate of potash (MOP) containing 50% K^+ while Magnesium fortification is done using commercially available fertilizer Magnesium Chloride ($MgCl_2$) whose Mg^{2+} content is 27%. The 18 FRP circular tanks (500L) with one control C (clear water) and five treatments (with different C/N ratios) T1 (5:1), T2 (10:1), T3 (15:1), T4 (20:1) and T5 (25:1) were filled with fortified 15 ppt inland ground saline waters upto 400 L each. The constant aeration provided to the experiment unit by air blower, through plastic tubing and air diffusers that

maintained the bio floc present in the tanks in suspension and distributed throughout the water column. Flocs inoculum was developed (4) by adding 20g of pond bottom soil was collected from CIFE centre Rohtak, India in well aerated water (1L) containing $10mg\ L^{-1}$ ammonium sulphate (NH_4SO_4) and $400mg\ L^{-1}$ of carbon sources (Rice Bran) in (5L) glass tanks after 48 hours, the inoculum was distributed equally into the already prepared experimental tanks. The tanks were kept well aerated for 10 days to ensure optimum floc production. After optimum floc produced in experimental tanks the 60/M2 SPF juveniles *Litopenaeus vannamei* (avg wt. 3.37 ± 0.03 gm) were stocked. The SPF juveniles obtained from a commercial shrimp hatchery, Andhra Pradesh, India.

Input C/N ratios were calculated based on the carbon nitrogen contents of the feed and the carbon content of the Rice bran. The nitrogen content of feed estimated by the Kjeldahl method and accordingly each gram of feed, carbon source as Rice bran added into each treatment tank for maintain the C/N ratio. The C/N ratio of water based solely on its total ammonia nitrogen (TAN) concentration (18). On average 75% of the feed nitrogen ends up in the water by ammonification of unutilised feed and excretion (21) Since the final disposal of most ($\pm 75\%$) of the added organic nitrogen is the culture medium, as inorganic nitrogen, mainly as TAN (8), it seemed practical to consider TAN as a useful index to estimate the concentration of total organic nitrogen in water. Accordingly, the concentrations of TAN of culture waters were determined weekly with a spectrophotometer at 640 nm by the phenate method (3). The determination of the required amount of carbon to reduce the total ammoniacal nitrogen was calculated as follows (27). Correction (g) = (TAN) x C:N x EF x volume of tank (L) /1000

Where [TAN] = Total ammoniacal nitrogen concentration (mg/L), C/N = C/N ratio and

EF = equivalence factor (Rice bran 2.31). All tanks were aerated and mixed continuously using air stones. Water temperature of the experimental tanks was maintained at around $26^\circ C$ during the culture period. No water was exchanged during the experimental period and only freshwater was added to compensate for evaporation losses and maintain the salinity. The photoperiod was maintained on a 12:12 hour light-dark cycle.

Determination of water quality parameters

The water quality parameters like temperature, P^H and salinity were measured using a digital thermometer (TC-902, Agrawal Electronics, Mumbai) water P^H was measured using portable P^H meter (P^H meter, Hanna Instrument, Italy) salinity was measured using hand-held Refractometer (Atago S/Mill-E, Japan). Dissolved oxygen, Total alkalinity, total ammonia-N (TAN), nitrite-N (NO_2N) and nitrate-N (NO_3N) were analyzed by following the procedures described in (3).

Growth performance

After end of experiment, tanks were drained and shrimp were collected. Shrimp from each tank were counted and total wet weight was determined. The growth parameters like final weight gain, feed conversion ratio (FCR), feed efficiency ratio (FER) protein efficiency ratio (PER) and specific growth rate (%) (SGR), as follows. Weight gain = $(FW - IW) \times 100 / IW$, FCR = Feed given (DW) / body weight gain (WW), FER = $1 / FCR$, SGR (%) = $[\ln(FW) - \ln(IW)] / N \times 100$. Where FW = final weight, IW = initial weight, DW = dry weight, WW = wet weight, ln = natural log and N = number of culture days.

Enzyme activity analysis

The digestive enzymes activity was evaluated after completion of the experiment, shrimp from each treatment groups were sacrificed for digestive enzyme analysis. The hepatopancreas and gut of the shrimp were dissected out, weighed and homogenized with 0.25 M chilled sucrose on wet basis (pH 7, 1:10 w/v) in ice cooled condition. The homogenate was centrifuged at 6000 rpm for 20min at 4 °C (Centrifuge 5417R, Eppendorf, Germany). After centrifugation, the floating top lipid layer was removed and the supernatant solution was divided as aliquots in 1.5 mL Eppendorf tubes. The samples were stored at -40 °C until analysis. Protease, Cellulase activity was evaluated according to standard methods (16) and amylase activity was measured by using iodine solution to reveal non-hydrolysed starch (14, 29). Lipase activity was determined based on the measurement of fatty acid release due to enzymatic hydrolysis of triglycerides in stabilized emulsion of olive oil (6, 16). Enzyme activities were measured as the change in absorbance using a spectrophotometer (UV-VIS spectrophotometer, GENEIX).

Data analysis

The statistical analysis was conducted using statistical package SPSS version 16.0A Means were separated at a significance level of $P < 0.05$ Comparison of difference in means among treatments was carried out using Duncan's multiple range test.

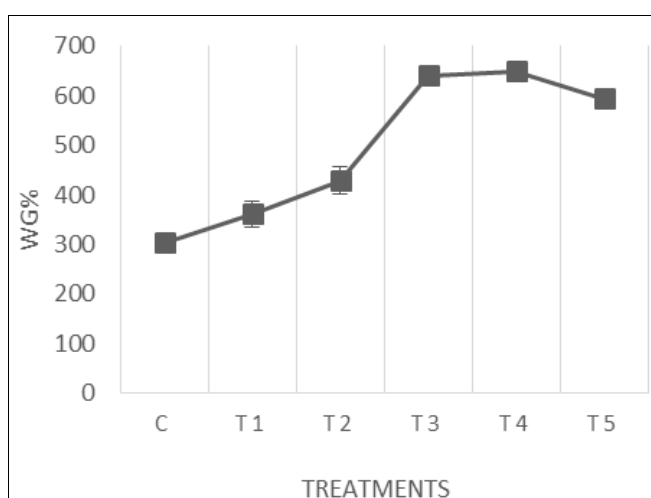
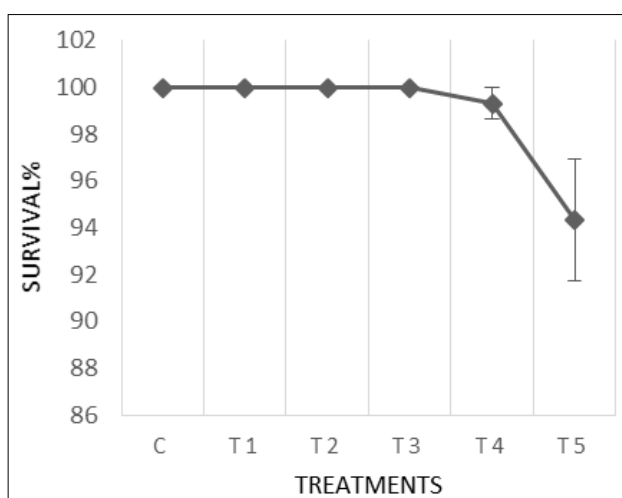
Results

In the present study the measured water quality in all experimental groups remained within recommended levels for shrimp culture throughout the 60 days experimental period. The growth performance and feed conversion (FCR, FER and PER) indices of *Litopenaeus vannamei* juveniles over the time period are presented in Table 1. In the present study on *Litopenaeus vannamei* has shown significantly higher weight gain % and SGR ($p < 0.05$) in treatment T3 ($641.07c \pm 12.55$ and $3.29d \pm 0.03$) and T4 ($650.00c \pm 5.88$ and $3.35d \pm 0.01$) respectively and in the treatment T1 and control group the growth rate was showed significantly similar ($p > 0.05$). A better FCR T4 ($1.04a \pm 0.01$), T3 ($1.12a \pm 0.02$) and T5 ($1.16a \pm 0.03$) was observed significantly lower from control ($2.23d \pm 0.04$) and T1 and T2. Similarly, significantly higher FER and PER was registered in T4 compared to control, T1 and T2 respectively. However, no significant difference in FER and PER was observed between T3 and T5. Highest 100% survival rate was reported in treatments T1, T2, T3 and control groups with 99.33% and 94.33% survival reported in T4 and T5 groups (Table:1, Fig.1). The digestive enzymatic activities of this study showed that the increasing levels of C/N ratios in bio floc improved ($p < 0.05$) the digestive enzymes activities in hepatopancreas and intestine like protease, amylase, lipase and cellulose in the treatment groups compared with control. (Table: 2 Fig.2). The lipase and cellulose activities in hepatopancreas and intestine were found significantly improved ($p < 0.05$) in both T3 and T4 treatments. (Table 3, Fig. 3).

Table: 1 Growth performance of *Litopenaeus vannamei* raised in bio floc Systems with different C/N ratios in inland ground saline water.

Treatments	Survival%	WG%	SGR%	FCR	FER	PER
C	100	303.74a \pm 6.32	2.32a \pm 0.02	2.23d \pm 0.04	0.45a \pm 0.09	1.28a \pm 0.03
T1	100	360.83a \pm 27.21	2.54b \pm 0.09	1.92c \pm 0.11	0.53b \pm 0.03	1.50b \pm 0.10
T2	100	428.34b \pm 27.95	2.76c \pm 0.09	1.61b \pm 0.10	0.62c \pm 0.03	1.79c \pm 0.01
T3	100	641.07c \pm 12.55	3.29d \pm 0.03	1.12a \pm 0.02	0.84d \pm 0.02	2.39d \pm 0.04
T4	99.33	650.00c \pm 5.88	3.35d \pm 0.01	1.04a \pm 0.01	0.96e \pm 0.01	2.74e \pm 0.02
T5	94.33	593.75cd \pm 19.34	3.22de \pm 0.04	1.16a \pm 0.03	0.87d \pm 0.02	2.47d \pm 0.07

Data expressed as Mean \pm SE, n=3; Mean values in the same column with different superscript differ significantly ($p < 0.05$). WG= Weight Gain SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, FER = Feed Efficiency Ratio, PER = Protein Efficiency Ratio.



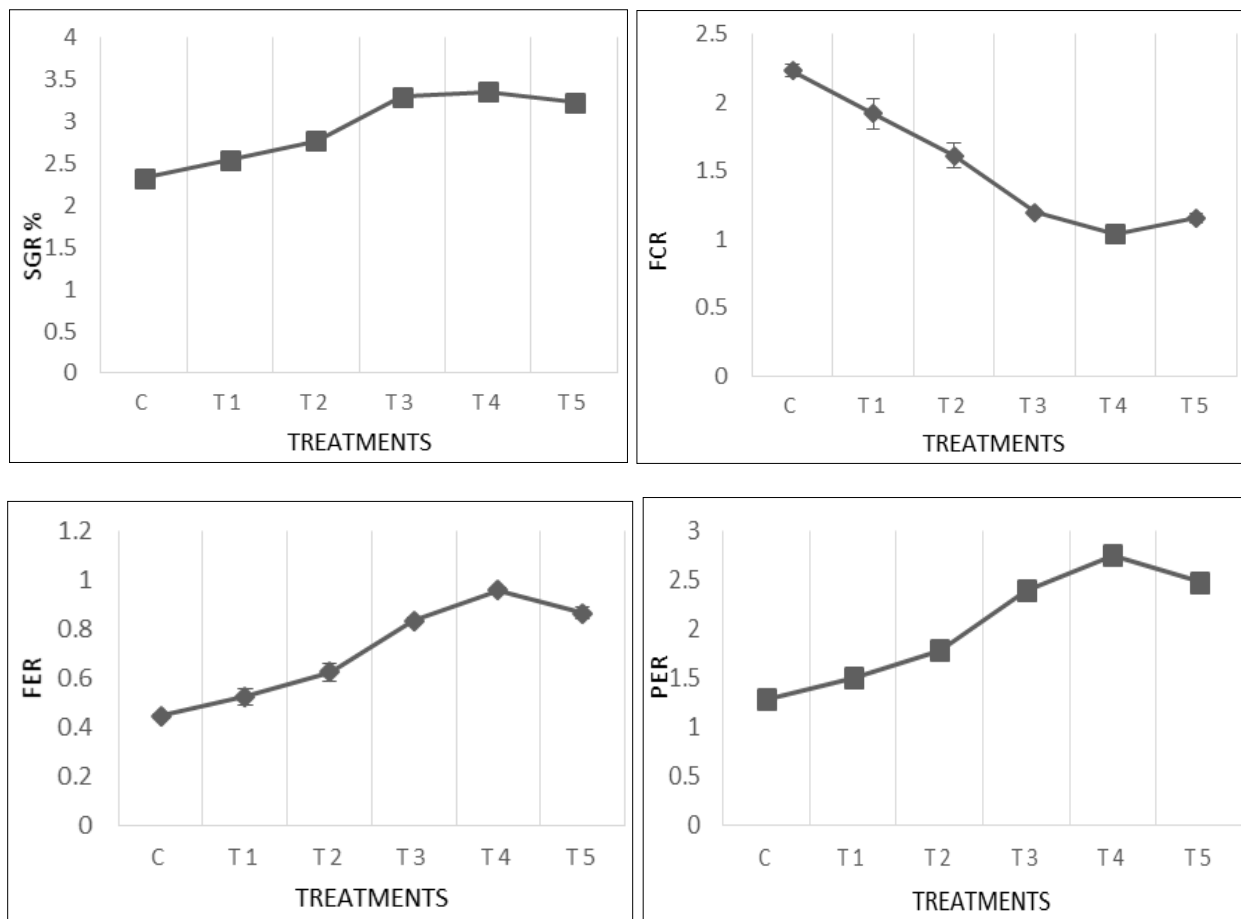


Fig 1: Growth performance of *Litopenaeus vannamei* raised in bio floc Systems with different C/N ratios in inland ground saline water. Error bars Represent (mean± SE).

Table 2: Digestive Enzyme Activities in Intestine of *Litopenaeus vannamei* raised in bio floc Systems with different C/N ratios in inland ground saline water.

Treatments	Protease	Amylase	Lipase	Cellulase
C	0.25a±0.03	0.35a±0.01	0.6a±0.02	0.034a±0.003
T1	0.28a±0.04	0.37a±0.01	0.65a±0.01	0.04a±0.003
T2	0.31a±0.08	0.4a±0.09	0.75b±0.02	0.06b±0.001
T3	0.35b±0.07	0.43ab±0.03	0.81bc±0.02	0.08c±0.009
T4	0.36b±0.07	0.46b±0.03	0.78b±0.02	0.08c±0.003
T5	0.35b±0.08	0.41a±0.02	0.78b±0.02	0.08c±0.001

Data expressed as Mean ± SE, n =3; Mean values in the same column with different superscript differ significantly (p < 0.05).

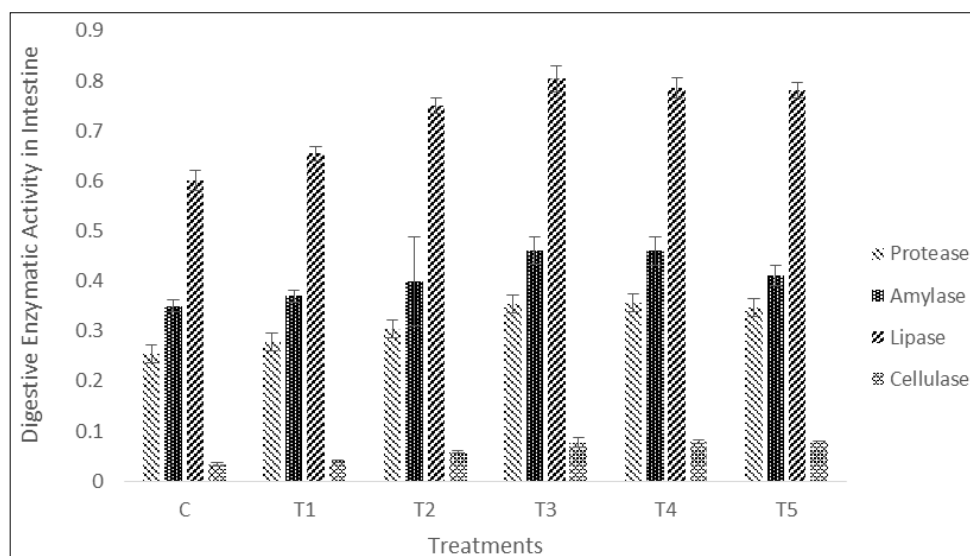


Fig 2: Digestive Enzymatic Activity in Intestine of *Litopenaeus vannamei* raised in bio floc Systems with different C/N ratios in inland ground saline water. Error bars Represent (mean± SE).

Table 3: Digestive Enzyme Activities in Hepatopancreas of *Litopenaeus vannamei* raised in bio floc Systems with different C/N ratios in inland ground saline water.

Treatments	Protease	Amylase	Lipase	Cellulase
C	0.25a±0.05	0.32a±0.09	0.83a±0.03	0.2a±0.03
T1	0.33a±0.06	0.51b±0.09	0.93a±0.09	0.35a±0.02
T2	0.48a±0.04	0.58b±0.15	0.98ab±0.23	0.45a±0.03
T3	0.65b±0.05	0.75c±0.15	1.04b±0.15	0.74b±0.02
T4	0.68b±0.06	0.74c±0.15	1.05b±0.09	0.78b±0.05
T5	0.74b±0.04	0.71c±0.15	0.94a±0.07	0.76b±0.04

Data expressed as Mean ± SE, n=3; Mean values in the same column with different superscript differ significantly ($p < 0.05$).

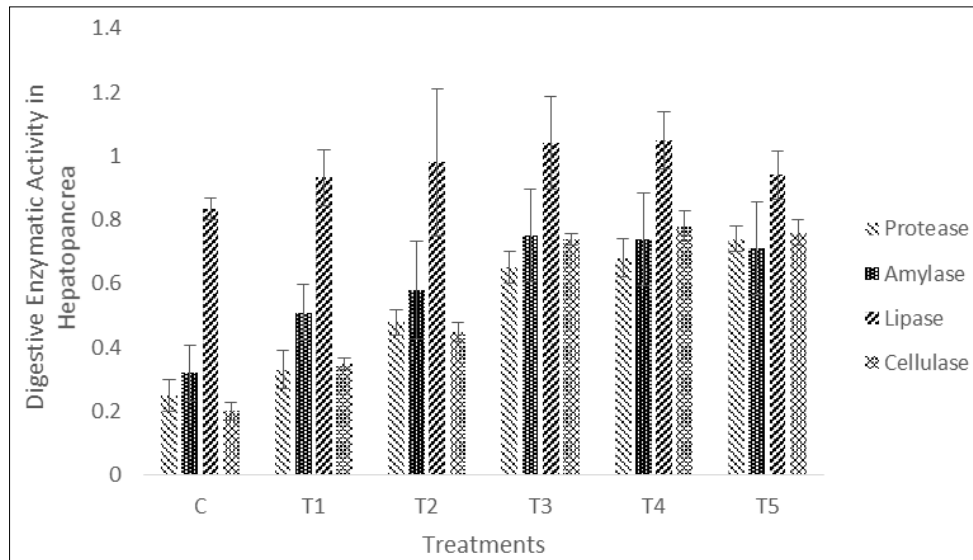


Fig 3: Digestive Enzymatic Activity in Hepatopancreas of *Litopenaeus vannamei* raised in bio floc Systems with different C/N ratios in inland ground saline water. Error bars Represent (mean± SE)

Discussion

In present study, rice bran was used as carbohydrate source for its easy availability and production of good quality floc by this high carbon source addition recorded higher weight gain, specific growth rate, FER, PER and lower FCR with respect to low or without carbon source added in control groups. In the earlier reports bio floc relies over the growth of heterotrophic microbes existing within the culture system, by increasing the C/N ratio (5, 13) microorganisms in bio floc not only detoxifies the nutrients, but also recycle those nutrients and used in animal growth by improving feed conversion ratios (17, 28). Similarly managing bio floc concentration could significantly improve shrimp growth rate, FCR, and biomass production (24). The C/N ratio of the aquatic ecosystem is above 10 should result in efficient ammonia assimilation (4, 10). The efficiently assimilate ammonia convert into bacterial biomass; however, the nitrogen they assimilate into biomass is entered into aquatic food chain. One of the possible reason for low survival compare to control and low C/N ratios with high C/N ratios is that the aeration requirements is more in these groups for respiration, mixing and maintaining the floc in suspended position in water column. In higher C/N ratios, the heterotrophic bacteria compete for available oxygen and space in the bio filters (19). Similarly bio floc with the high C/N ratios increases the oxygen demand due to the fast assimilation of TAN by heterotrophic bacteria, this aspect of high C/N ratios can affect the species sensitive to different levels of dissolved oxygen (15, 23, 26). The increased digestive enzyme activities in T3 and T4 might have enhanced the digestion and nutrient absorption which might have contributed significantly higher ($p < 0.05$) growth rate in

these treatment groups as compare to control group. The bio floc based system, reported significantly higher digestive enzyme activities and better growth performance in shrimps (2, 30). Bio floc supplementation at the 12% level shows decline trend in growth rate, FCR and digestive enzyme activities as compared with low level of bio floc supplementation (2). Similarly the growth rate of fishes was decreases at higher level of microbial supplementation (1). However, the growth performance and digestive enzyme activities in shrimps in T5 (C/N 25) group was compared to low C/N ratio do not shows growth retardation effects in shrimps but the C/N ratios blow (20:1) in bio floc shows good in growth performance and digestive enzyme activities in shrimp raised in inland ground saline water.

Conclusion

The present study demonstrated that development of bio floc with C/N ratios (15:1 and 20:1) in inland ground saline water shows good growth performance and digestive enzyme activities in *Litopenaeus vannamei*. An important potential conclusion of this work is to encourage shrimp farmers in inland saline areas to use low cost carbon source as rice bran with appropriate C/N ratios for development of bio floc to minimizing the water exchange in inland saline areas for shrimp farming.

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References

1. Ajiboye, OO, Yakubu AF, Adams TEA, Perspective on the ingestion and nutritional effects of feed additives in farmed fish species. *World J Fish Mar. Sci.* 2012; 4:87-101
2. Anand PSS, Kohli MPS, Kumar S, Sundaray JK, Roy SD, Venkateshwarlu G *et al.* Effect of dietary supplementation of bio floc on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture.* 2014; 418:108-115.
3. APHA. Standard methods for the examination of water and wastewater. In: Clesceri LS, Greenberg AE, Eaton AD (eds) American Public Health Association, American Water Works Association and Water Environment Federation. United Book Press, Washington DC, 1998.
4. Avnimelech, Y. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture.* 1999; 176, 227-235.
5. Azim ME, Little DC, Bron JE. Microbial protein production in activated suspension tanks manipulating C/N ratio in feed and the implications for fish culture. *Bio resource Technology.* 2008; 99:3590-3599.
6. Borlongan IG. Studies on the digestive lipases of milkfish, *Chanos chanos*. *Aquaculture.* 1990; 89:315-325.
7. Crab R, Chielens B, Wille M, Bossier P, Verstraete W. The effect of different carbon sources on the nutritional value of bio flocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture Research.* 2010; 41:559-567.
8. Crab R, Defoirdt T, Bossier P, Verstraete W. Bio floc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture.* 2012; 356-357, N. (1)351-356.
9. Davis DA, Saoud IP, Boyd CE, Rouse DB. Effects of potassium, magnesium, and age on growth and survival of *Litopenaeus vannamei* post-larvae reared in inland low salinity well waters in west Alabama. *Journal of the World Aquaculture Society.* 2005; 36: 403-406.
10. Ebeling JM, Timmons MB, Bisogni JJ Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture.* 2006; 257:346-358.
11. Gong H, Jiang DH, Lightner DV, Collins C, Brock DA. dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. *Aquaculture Nutrition.* 2004; 10:227-236.
12. Hargreaves JA. Photosynthetic suspended-growth systems in aquaculture. *Aquaculture Engineering.* 2006; 34:344-363.
13. Hari B, Kurup BM, Varghese JT, Schrama JW, Verdegem MCJ. Effects of carbohydrate addition on production in extensive shrimp culture systems. *Aquaculture.* 2004; 241:179-194.
14. Jiang CK. *Manual of Enzyme Activity Measuring.* Science and Technology Press, Shanghai, 1982.
15. Jimenez-Ojeda YK, Collazos-Lasso LF, Arias-Castellanos JA. Dynamics and use of nitrogen in Bio floc Technology - BFT. *AACL Bio flux.* 2018; 11(4):1107-1129.
16. Jin ZL. *The Evaluation Principle and Method of Functional Food.* Beijing Publishers, Beijing, 1995.
17. Ju, ZY, Forster IP, Dominy WG. Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). *Aquaculture.* 2009; 292:237-243.
18. Martins RB, Vale DA, Rebouças VT, Carmo MV. Imbalanced C/N-controlled, periphyton-based system has hampered tilapia growth in stagnant experimental tanks. *Acta Scientiarum Technology.* 2014; 36(2):229-235.
19. Michaud L, Blancheton JP, Bruni V, Piedrahita R. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquac. Eng.* 2006; 34:224-233.
20. Mishra JK, Samocha TM, Patnaik S, Speed M, Gandy RL, Ali AM. Performance of an intensive nursery system for the Pacific white shrimp, *Litopenaeus vannamei*, under limited discharge condition. *Aquacultural Engineering.* 2008; 38:2-15.
21. Piedrahita, R.H. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture.* 2003; 226:35-44.
22. Ravichandran P. Shrimp farming status, issues and strategies for sustainable development. *Manual - Summer School on Advances in Aquaculture Nutrition and Feed Processing Technology CIBA, Chennai.* 2011; 36-41.
23. Ray AJ, Lotz JM, Comparing a chemoautotrophic-based bio floc system and three heterotrophic-based systems receiving different carbohydrate sources. *Aquacultural Engineering.* 2014; 63:54-61.
24. Ray AJ, Lewis BL, Browdy CL, Leffler JW. Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant based feed in minimal-exchange, superintensive culture systems. *Aquaculture.* 2010; 299:89-98.
25. Saoud IP, Davis AD, Rouse DB. Suitability of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture.* 2003; 217:373-383.
26. Schweitzer R, Arantes R, Costódio PFS, do Espírito Santo CM, Arana LV, Seiffert WQ, Andreatta ER/ Effect of different bio floc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. *Aquacultural Engineering.* 2013; 56:59-70.
27. Serra FP, Gaona CAP, Furtado P, Poersch LH, Wasielesky JR. Use of different carbon sources for the bio floc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. *Aquaculture International.* 2015; 23(6).
28. Wasielesky WJ, Atwood HI, Stokes A, Browdy CL. Effect of natural production in brown water super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture.* 2006; 258:396-403.
29. Worthington V. *Worthington Enzyme Manual.* Enzymes and Related Bio chemicals Worthington Chemical, New Jersey, US, 1993.
30. Xu WJ, Pan LQ. Effects of bio flocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture.* 2012; 356:147-152.