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Effects of Biofloc Technology (BFT) on Growth of Speckled Shrimp (*Metapenaeus monoceros*)

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ABSTRACT

The effects of biofloc technology (BFT) on growth and hepatopancreas histology of speckled shrimp *Metapenaeus monoceros* (initial weight: 8.32±0.69 g) were investigated for 30 days. The trial was conducted in fiberglass tanks (45 L) with biofloc (zero-water exchange) and without biofloc (water exchange 50% day⁻¹). Different carbon sources (corn starch: CS and glycerine: G) and mannan oligosaccharides (MOS) supplementation (with and without BFT) were tested in triplicate groups. At the end of the experiment, better growth performances, feed conversion ratio, and specific growth rates were detected in shrimps reared in BFT (CS, G, CS+MOS, G+MOS) groups. Measured water quality parameters (dissolved oxygen, pH, temperature, salinity, ammonium-NH₄ and nitrate-NO₃) did not differ between BFT and control groups. The total number of bacteria count of shrimp reared in the biofloc groups were higher (4.9x10⁶±8.5x10⁴ CFU mL⁻¹) than that of in the without biofloc (3.7x10⁶±5.4x10⁴ CFU mL⁻¹) groups. In this study BFT had no negative effects on hepatopancreatic tissue by histological assessment. It is suggested that corn starch as a carbon source in BFT with 3 g kg⁻¹ MOS supplementation could be applied as healthy growth enhancer in speckled shrimp culture.

Keywords: Corn starch; Glycerol; Growth; Histology; Mannan oligosaccharides; *Metapenaeus monoceros*

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1. Introduction

Aquaculture plays a crucial role for supporting the world's needs of protein (Jackson 2007; Kuhn et al 2010). According to FAO and World Bank 2030-2050 projections the production capacity of aquaculture should be doubled because of the population increases and global warming. The report also proposes that agriculture will

be practiced in marine environment. In order to achieve this successful and secure food production, the industry will obviously need to develop new technologies that will increase sustainable economic profitability and environmental concerns. Intensive aquaculture applications work with great amounts of clean water exchange ratios to sustain acceptable water quality (Wang 1990; Hopkins

et al 1993; Moss et al 1999; Mishra et al 2008). However, due to environmental concerns like conservation of water sources, the requirement for more ecologically sound management and aquaculture practices is also growing steadily (Emerenciano et al 2012).

It is known that microorganisms have an important influence on nutrition cycle, water quality, and nourishment of cultured species (Emerenciano et al 2011). Biofloc technology is a technique utilized to increase water quality and create extra protein resources by adding an external carbon source into environmentally friendly aquaculture systems. The sustainable and recyclable food production approach based on such a system is dependent on the growth of microorganisms. Biofloc technology today has been implemented with minimum or zero water exchange in culture systems. Moreover, it is possible to reduce feed consumption by recycling all the waste for the edible food sources by BFT for cultured species (Kaya & Genc 2018).

Studies investigating alternative/new species for aquaculture are vitally important to gain insight regarding market diversification and minimization of food shortage. Speckled shrimp, *Metapenaeus monoceros*, is the one of highly demanded penaeid shrimp in fish market of Mediterranean and Indo-Pacific region and is considered as an alternative species in aquaculture. The main objective of the current study was to determine the growth parameters, survival, and hepatopancreas histology of speckled shrimp with BFT (in zero water exchange) and without BFT application (50% day⁻¹ water exchange).

2. Material and Methods

2.1. Experimental design and culture conditions

The study was conducted at the Fisheries Research Unit (Faculty of Agriculture, Ankara University), Ankara, Turkey. The trial was conducted in fiberglass tanks (45 L) with biofloc (zero-water exchange) and without biofloc (water exchange 50% day⁻¹).

Shrimps used in the study were obtained from the Faculty of Marine Sciences and Technology of Iskenderun University and transported to the Ankara University.

Effects of biofloc treated with two different carbon sources (corn starch: CS and glycerine: G) and mannan oligosaccharide (MOS) as a prebiotic on growth of speckled shrimp were investigated. In the experiment, 90 shrimps (8.32±0.69 g) were used in four different biofloc groups (CS, G, CS+MOS, G+MOS) and two groups without biofloc (MOS and Control) for 30 days. Each treatment was randomized with three replicates (5 shrimps/tank). Each biofloc was produced with different carbon sources (by starting before the 15 days of experiment and maintained with daily carbon addition, at 28.82-29.33 ppt salinity and 25.10-25.45 °C water temperature). Biofloc volume was controlled with imhoff to ensure that it remains at the level of 10-20 mL L⁻¹ in biofloc groups. Shrimp were fed with 45% crude protein and 20% lipid marine fish commercial diet (Sibal LTD. Sinop, Turkey) at 3% of the estimated biomass (three times a day; 08.00, 13.00, 18.00) throughout the experiment. In the trial, a 12-h photoperiod was sustained by using fluorescent lighting. Corn starch and glycerine were added daily after feeding to maintain C:N as 15 during the study to ensure heterotrophic bacterial growth.

2.2. Water quality parameters

Throughout the experimental period, pH, temperature, salinity, and dissolved oxygen were measured daily using a multi-parameter instrument (YSI® 556, YSI Inc., Yellow Springs, OH, USA) at 08:30 hours in all tanks. Ammonium (NH₄) and nitrate (NO₃) were monitored once a week according to standard methods (APHA 1998).

2.3. Growth parameters

At the end of the study, the final weight, daily weight gain, feed conversion ratio (FCR), specific growth rate (SGR), and survival rate of six treatments were calculated as indicated below.

$$\text{Weight gain (g/shrimp)} = \text{Final weight (g)} - \text{Initial weight (g)} \quad (1)$$

$$\text{Feed conversion ratio (FCR)} = \text{Total feed given (g)} / \text{Weight gain (g)} \quad (2)$$

$$\text{Specific growth rate (SGR; \% / day)} = ((\ln(\text{Final weight}) - \ln(\text{Initial weight})) / \text{days}) \times 100 \quad (3)$$

$$\text{Survival rate (\%)} = (\text{Final number of shrimp}) / (\text{Initial number of shrimp}) \times 100 \quad (4)$$

2.4. Histology

Three shrimp samples from each triplicate group were anaesthetized with 5 mg L⁻¹ quinaldine (Sigma Chemical Company, St. Louis, Missouri, USA) (Genc et al 2007), the carapax were opened, and hepatopancreas tissue were removed by dorso-ventral incision. The hepatopancreas samples were fixed for 24 h in 10% buffered formaline solution. After dehydration by passing tissues through a series of ethanol solutions (70, 85 and 98%) and clarification by two series of xylene, the samples were vacuum embedded in paraffin at 59±1 °C. The microtome sections (4-5 µm, Thermo Shandon, Germany) were stained for morphological purposes with haematoxylin and eosin (H & E) and then were analysed on microphotographs (Leica CM40) (Roberts & Smail 2004).

2.5. Total bacterial counts

Water samples from the culture tanks were stored in sterile glass bottles (50 mL) and analysed for total heterotrophic bacteria (THB). The preparation of the sample dilutions and bacteriological assays of the water were conducted separately and their averages were computed using the method described by APHA (2005). Total viable bacterial count from tank water was determined by the spread-plate technique on Plate Count Agar (PCA) with 2% NaCl. In each sample, serial dilutions were prepared in macro dilution tubes with concentrations ranging between 10⁻¹ to 10⁻⁸ and were incubated at 37 °C for 48 hours. After the incubation period, the colonies were counted by eye and CFU values (CFU mL⁻¹) were calculated. Cultures containing less than 30 colonies and more than 300 colonies were not considered in the analyses.

The CFU mL⁻¹ values were calculated using the formula;

$$\text{CFU mL}^{-1} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of culture plate} \quad (5)$$

2.6. Statistical analysis

Statistical analysis was performed using SPSS, IBM, Statistics 23.0 for Windows. Analysis of variance (ANOVA) was used on shrimp performance and water quality parameters data to analyse the effect of BFT. The α level of 5% was used for all tests.

3. Results and Discussion

Water quality values are listed in Table 1. There were significant differences ($P < 0.05$) among groups with regards to dissolved oxygen and pH parameters. Levels of dissolved oxygen and pH ranged from 6.02 to 6.29 mg L⁻¹ and 7.73 to 7.96, respectively. The concentrations of ammonium (NH₄) and nitrate (NO₃) were found similar for all groups ($P > 0.05$). The total number of bacteria count of shrimp reared in the biofloc groups were higher ($4.9 \times 10^{-6} \pm 8.5 \times 10^{-4}$ CFU mL⁻¹) than that of in the no-biofloc ($3.7 \times 10^{-6} \pm 5.4 \times 10^{-4}$ CFU mL⁻¹) groups ($P < 0.05$) (Table 2).

Shrimp growth performance is presented in Table 3. Final weights of shrimp reared in CS+MOS group were significantly higher ($P < 0.05$) than that of shrimp reared in MOS and C groups. There was no significant difference among the biofloc treated groups in terms of final weights ($P > 0.05$). The highest weight gain (g) was obtained in group CS+MOS and the lowest value was observed in the control group ($P < 0.05$). The best feed conversion ratio (FCR) of shrimp was observed in group CS+MOS and the highest FCR was calculated from the control group ($P < 0.05$). The specific growth rate (%/day) and survival rate (%) of shrimp were higher in the biofloc treatments compared to the MOS and C, but no significant differences were detected among all groups ($P > 0.05$).

Table 1- Water quality parameters of speckled shrimp culture environments

Water parameters	Biofloc groups				Without biofloc groups	
	CS	G	CS+MOS	G+MOS	MOS	Control
DO (mg L ⁻¹)	6.29±0.42 ^a	6.06±0.26 ^{ab}	6.02±0.68 ^b	6.07±0.35 ^{ab}	6.06±0.23 ^{ab}	6.15±0.41 ^{ab}
pH	7.95±0.18 ^b	7.73±0.45 ^a	7.88±0.19 ^b	7.96±0.14 ^b	7.95±0.10 ^b	7.94±0.10 ^b
T (°C)	25.15±0.50	25.30±0.55	25.10±0.70	25.45±0.50	25.20±0.64	25.29±0.72
Salinity ppt	29.33±0.76	29.07±0.87	29.03±0.85	28.93±0.90	28.82±0.86	29.13±1.22
NH ₄ (mg L ⁻¹)	0.18±0.01	0.23±0.05	0.14±0.01	0.15±0.01	0.15±0.01	0.14±0.01
NO ₃ (mg L ⁻¹)	0.21±0.10	0.21±0.10	0.21±0.10	0.25±0.09	0.23±0.09	0.25±0.09

CS, Corn starch; G, Glycerol; MOS, Mannan oligosaccharides. Different superscripts in the same line indicate significant differences (P<0.05)

Table 2- Results of total aerobic bacteria counts

Total aerobic bacteria	Biofloc groups				Without Biofloc groups	
	CS	G	CS+MOS	G+MOS	MOS	Control
TBC CFU (mL ⁻¹)	4.8x10 ^{-6±} 5.8x10 ^{-4b}	4.8x10 ^{-6±} 5.8x10 ^{-4b}	5.0x10 ^{6±} 5.8x10 ^{-4c}	5.0x10 ^{-6±} 5.8x10 ^{-4c}	4.1x10 ^{-5±} 3.3x10 ^{-4a}	3.3x10 ^{-5±} 1.5x10 ^{-4a}
Mean CFU (mL ⁻¹)	4.9x10 ^{-6±} 8.5x10 ⁻⁴				3.7x10 ^{-6±} 5.4x10 ⁻⁴	

CS, Corn starch; G, Glycerol; MOS, Mannan oligosaccharides. Different superscripts in the same line indicate significant differences (P<0.05)

Table 3- Growth parameters

Growth parameters	Biofloc groups				Without Biofloc groups	
	CS	G	CS+MOS	G+MOS	MOS	Control
IW (g)	8.33±0.69 ^a	8.30±0.79 ^a	8.36±0.83 ^a	8.36±0.81 ^a	8.32±0.74 ^a	8.35±0.75 ^a
FW (g)	10.99±0.9 ^{ab}	10.89±0.77 ^{ab}	11.51±0.75 ^b	10.93±0.93 ^{ab}	10.65±1.06 ^a	10.44±0.83 ^a
WG (g)	2.64±0.62 ^{ab}	2.63±0.32 ^{ab}	3.15±0.28 ^b	2.56±0.36 ^{ab}	2.36±0.69 ^{ab}	2.08±0.36 ^a
FCR	2.81±0.63 ^{ab}	2.89±0.32 ^{ab}	2.38±0.26 ^a	2.92±0.49 ^{ab}	3.24±0.90 ^{ab}	3.59±0.73 ^b
SGR (%/day)	0.92±0.21 ^a	0.92±0.09 ^a	1.07±0.11 ^a	0.89±0.13 ^a	0.83±0.24 ^a	0.75±0.16 ^a
SR (%)	93.33 ^a	86.67 ^a	93.33 ^a	93.33 ^a	86.67 ^a	86.67 ^a

CS, Corn starch; G, Glycerol; MOS, Mannan oligosaccharides. Different superscripts in the same row indicate significant differences (P<0.05) IW: Initial weight, FW: Final weight, WG: Weight gain, FCR: Feed conversion ratio, SGR: Specific growth rate, SR: Survival rate

Hepatopancreas morphology was normal and did not differ among the groups (Figure 1). Different cell types were recognized but the structures were normal.

There are limited studies on growth of speckled shrimp, *M. monoceros*. In this study, water quality parameters (DO, pH, temperature, and salinity) were measured in the recommended range of

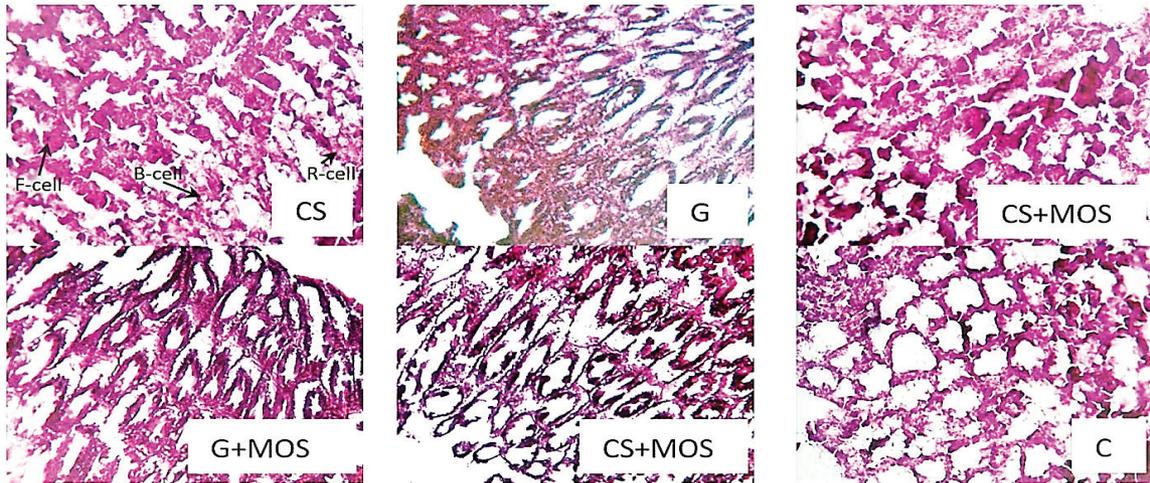


Figure 1- Speckled shrimp hepatopancreas tissue sections (10x, H & E)

marine shrimp culture with BFT. Water quality parameters observed in the current study were found to be similar to the study by Rahman et al (2010) for speckled shrimp. In BFT, where water exchange is limited, the inhibition of accumulation of nitrogenous toxic compounds may be possible by maintaining high C:N ratio and the consumption of ammonia by the microbial community (Avnimelech 1999; Kuhn et al 2010; Emerenciano et al 2012). In this study, ammonium and nitrate levels did not differ statistically between BFT and without BFT groups. Results reveal that the use of corn starch and glycerol as the carbon source and the maintenance of the optimum C:N ratio can sustain the low level of toxic nitrogen compounds in the culture environment. The mean total number of bacteria counts in the BFT groups were observed in similar ranges compared to the ranges reported in previous study ($4.23\text{--}4.55 \times 10^{-6}$ CFU mL⁻¹) by de Paiva Maia et al (2016). However, bacterial count ranges not only in BFT groups but also in without BFT groups of the present study were higher (245.67×10^{-8} CFU mL⁻¹) compared to the study by Anand et al (2014). This discrepancy may be explained by the low salinity (13.67-14.29 ppt) conditions used in the previously mentioned study.

Digestive glands consist of diverticula of the intestine and the main functions of the hepatopancreas are absorbing nutrients, storing lipids, and producing digestive enzymes (Johnson 1980; Genc et al 2007). According to the literature on physiology of digestion, absorption plays an important role in lipoprotein metabolism and could be used to monitor nutritional value of shrimp diets. In this study, the speckled shrimp reared with BFT and without BFT conditions fed on commercial marine fish diet with or without MOS supplementation showed similar and normal morphology.

It is argued that it can be possible to obtain better yield from a unit of area by using BFT in aquaculture. In our experiment, the growth parameters (the final weight, daily weight gain, feed conversion ratio, specific growth rate, and survival rate) were higher in BFT groups than the shrimp in the without BFT groups. The final weight of shrimp treated with CS+MOS was statistically higher than that of shrimp reared in the control group ($P < 0.05$). These results revealed that BFT increased growth parameters in shrimp culture in parallel with previous studies (Krummenauer et al 2011; Emerenciano et al 2011; Xu & Pan 2012; Emerenciano et al 2012; Kumar et al 2017; Lara et al 2017).

4. Conclusions

Although previous studies focused on the effects of different carbon sources in BFT on growth parameters (Crab et al 2012; Emerenciano et al 2013), the current study investigated the effect of prebiotic supplementation with two different carbon sources. In conclusion, the combined use of corn starch and mannan oligosaccharide in biofloc technology could be considered as a productive application for shrimp culture. In addition, BFT application on speckled shrimp larvae is recommended for future studies. This study is the first record on speckled shrimp *M. monoceros* culture with BFT treatment.

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