

Research Article

Effect of adding vegetable substrates on *Penaeus vannamei* pre-grown in biofloc system on shrimp performance, water quality and biofloc composition

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ABSTRACT. The effect of adding vegetable substrates: wheat bran (WB), oat bran (OB) and amaranth seeds (AS) on water quality, biofloc composition and production response of *Penaeus vannamei* intensively pre-grown, was evaluated during five weeks. Water quality parameters (temperature, pH, salinity and dissolved oxygen) were monitored twice a day; nitrogenous compounds (TAN, NO₂-N, NO₃-N) and the total suspended solids (TSS), were recorded weekly. Weight gain and survival of juveniles were monitored each week. Chemical proximate composition of biofloc and content of heterotrophic bacteria were recorded at the end of the trial. No significant differences were observed for any of the water quality parameters or nitrogenous compounds. The TSS was higher in treatments with substrates compared to the control. The treatment WB showed the highest content of heterotrophic bacteria. Organic matter in the bioflocs ranged from 58.3 to 62.4%, proteins from 33.4 to 37.6%, lipids from 0.4 to 0.5% and carbohydrates from 17.1 to 25.5%, without significant differences in any of them among treatments. The mean final weight varied from 1.10 to 1.38 g, and specific growth rate from 5.83 to 6.43% d⁻¹, with the highest values in treatment OB. Final survival ranged from 93.7 to 98.8% without significant differences among treatments and the control. The feed conversion ratio varied from 0.92 to 1.20 with the lowest value in OB compared to the control. The results suggest that supplementation of substrates does not have a negative effect on water quality, but have positive effects on the productive response of shrimp during their pre-grown, being oat bran the most adequate.

Keywords: *Penaeus vannamei*; biofloc; shrimp nutrition; vegetable substrate; biofloc composition; aquaculture

INTRODUCTION

During 2014 the world production of aquatic animals from aquaculture reached over 73.8 million tonnes with a gross value around USD 160,200 million (FAO, 2016), despite the severe problems the activity has faced, mainly related to the continuous presence of epizootics such as the WSSV and the EMS. Only in the state of Sonora, northwestern Mexico, the production during 2013 decreased from 80,000 to less than 35,000 t.

One of the diverse alternatives implemented worldwide to face the problem is the use of biosecure closed or semi-closed systems with recirculation and low or zero water exchange (González-González *et al.*, 2009; Zhou & Hanson, 2017). Most of these systems

are now incorporating the biofloc technology (BFT) which contributes to maintain a good water quality (Esparza-Leal *et al.*, 2015; Bossier & Ekasari, 2017), complement shrimp nutrition (Xu *et al.*, 2012; Maciel *et al.*, 2018) and improve their physiological condition and immune response (Zhang *et al.*, 2017).

The BFT effectively utilizes the microbial biomass associated with fixing or floating substrates and has demonstrated some advantages over other traditional technologies in aspects such as the reduction of required water and pathogens control (Rego *et al.*, 2017; Moreno-Arias *et al.*, 2018).

The bioflocs can be formed without requiring substrate inputs because several microbes can promote a biofouling process using practically any floating par-

ticle from the water column (feces, molts, unconsumed feed and other); however, bioflocs can proliferate faster if these substrates are provided at the adequate rates. In this context, it is necessary to consider the total amount of suspended solids without surpassing safe levels that could cause problems in the system (Gaona *et al.*, 2016).

The proximate biochemical composition of the floc biomass may vary widely depending on factors such as the time of culture (maturation), farming conditions, organisms farmed, carbon and nitrogen rate and source, and substrate used (Martínez-Córdova *et al.*, 2015).

Vegetal fibers contain materials non-digestible by animal enzymes, but they can be used by microorganisms as nucleation sites to begin a biofouling process, using the same substrate as carbon source. This kind of material is mainly constituted by structural polysaccharides, including cellulose, hemicellulose, beta-glucans, pectin, mucilage, and gums (Cabrera & Cárdenas, 2006). The structural differences among them determine their physical and chemical properties (Meyer, 2004), and their ability to serve as a substrate for microbial bioflocs.

Diverse materials, especially from the vegetal origin, have been evaluated as nucleation sites for flocculation, including wheat bran (Becerra-Dorame *et al.*, 2012; Emerenciano *et al.*, 2012), brewery residues (Fugimura *et al.*, 2015), cane bagasse, and other. In most cases the proximate biochemical composition of the bioflocs can be considered appropriate to complement the nutrition of the farmed organisms, because these microbial masses registered protein levels ranging from 23 to >38% (Moreno-Arias *et al.*, 2018), and lipids from <1 to >8% (Wei *et al.*, 2016). The lipid contents of bioflocs are usually low; however, the proportion of PUFA and HUFA are high (Dauda *et al.*, 2018).

The present study was performed to evaluate the effect of adding three different vegetable substrates, on the proximate biochemical composition of the bioflocs, on the water quality of the system and the productive response of shrimp intensively farmed.

MATERIALS AND METHODS

The study was conducted during 36 days in the aquaculture unit of the Universidad Estatal de Sonora, sited at Navojoa, Sonora, Mexico. A single-factor and completely randomized experimental design with three replicates per treatment were performed. The treatments consisted of three different vegetable floating substrates added to the water column: wheat bran (WB), amaranth seeds (AS) and oat bran (OB), which were compared to the control (C) without substrate.

The experimental units were plastic tanks with an operative volume of 200 L. The marine water (36 of salinity) was filtered (5 μ), sterilized with chlorine (1 ppt) and neutralized with ammonium thiosulfate. One day before, of the beginning of the experiment, the tanks were filled up, and the levels of the main environmental variables were adjusted. The temperature of the water was maintained at around 28°C with Via Aqua titanium heaters (150 W, 110-120 V; thermostat included), the photoperiod was 12:12. Constant aeration was provided by an electric blower (Sweet water; Aquatic-Ecosystems 1/3 HP, 115 V) equipped with air filter, to achieve dissolved oxygen (DO) levels over 5 mg L⁻¹. During the experiment, the salinity was maintained by replacing evaporation losses with sterilized fresh water.

Each experimental unit was stocked with 200 postlarvae (1,000 postlarvae m⁻³) of *Penaeus vannamei* (12 postlarvae, with a mean weight of 12 mg). Postlarvae were fed three times a day with a commercial diet (Agribrand Purina 35% CP). The feeding rate was adjusted to 20% of the total biomass per day at the beginning of the trial, reducing it gradually up to 15% at the end. At days 1, 7 and 14, each unit was supplied with the corresponding substrate at a rate of 0.15 g L⁻¹ to promote the formation of bioflocs. To reach C:N rate around 12:1, in all treatments unrefined sugar as organic carbon source was added. In the calculation, we followed the indications of Avnimelech (2009), which consider the proximate composition of the pelleted feed.

During the substrate addition (days 1, 7 and 14) the proximate composition of the substrates were considered to maintain the C:N rate around 12:1. Therefore, sugar was limited in the substrate treatments. In the calculations, the proximate composition of substrates was considered as follow, wheat bran (protein 16.2%, lipid 0.4%, carbohydrates 30.0% and fiber 38.0%; Nascimento *et al.*, 2014); amaranth (protein 65.6%, lipid 0.9%, carbohydrates 7.7% and fiber 22.4%; Chen *et al.*, 1988); and oat bran (protein 5.5%, lipid 1.0%, carbohydrates 56.6% and fiber 26.4%; Nascimento *et al.*, 2014).

The main water quality variables were monitored twice a day. Temperature and DO by mean of a digital DO meter YSI model 55 and pH with a Denver digital pH meter. The total suspended solids were recorded each week by the method 8006 (Hach, 2007) using a spectrophotometer Hach DR/2800. The same apparatus was used to determine total ammonium nitrogen (TAN) by the salicylate method (855), nitrite by the diazotization method (8507), and nitrate by the reduction and diazotization method (8171); these measurements were done weekly.

At the end of the trial, samples of bioflocs from each experimental unit were collected by decantation using Imhoff cones; after that, biofloc matter was dehydrated in a digital Shell Lab stove at 70°C for 72 h. The proximate biochemical composition was evaluated as follows: moisture by difference of weight after dehydration; ash by calcination in a muffle and considering the weight difference; protein by the micro-Kjeldahl method following the technique 8075 of the Hach DR2800 spectrophotometer; lipids were measured by using a Soxtec Avanti 800 equipment, with petroleum ether as extractor solution. Carbohydrates (CHO) were calculated by difference according to the next equation:

$$\% \text{ CHO} = (100 - [\text{proteins} (\%) + \text{lipids} (\%) + \text{ash} (\%)]) / (100)$$

Samples of water from each experimental unit were collected at the end of the experiment, in sterilized plastic bags (Whirl-Pak^{MR}) to perform a bacteriological analysis by surface dispersion in marine agar (medium 226, DIFCO^{MR}) following the method of Greenberg *et al.* (1992). The plates were incubated at 30 ± 2°C for 24 h. The colonies were counted and expressed as colony-forming units per milliliter (CFU mL⁻¹).

A sample of 20 shrimp from each unit was collected weekly to assess the production parameters and weighed on an analytical balance Scientech (ZSA 210), to calculate the mean weight in each treatment. At the end of the trial, survival was recorded by counting the shrimp from each unit.

The specific growth rate was calculated as:

$$\text{SGR} = \frac{[\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}]}{\text{days of culture}} \times 100$$

The feed conversion ratio was estimated as:

$$\text{FCR} = \frac{\text{Consumed feed}}{\text{Weight gain}}$$

For statistical analyses, all data were submitted to probes of normality and homogeneity of variance. As they passed both probes, a one-way ANOVA was performed to detect differences among treatments. When differences were significant, a Tukey test was applied (Tukey, 1949) to order and rank means. These analyses were done by using the software Statistica 5.1 for Windows (Statsoft Inc.®).

RESULTS

No significant differences were found in the primary water quality variables among treatments (Table 1). Mean temperature ranged from 27.7 to 28.0°C, and dissolved oxygen varied from 5.0 to 5.3 mg L⁻¹. The pH recorded values from 8.1 to 8.2.

The total suspended solids were significantly higher in the treatments with substrates compared to the

control. The higher mean concentration was recorded in treatment amaranth seeds (AS) (130 mg L⁻¹) and the lowest in the control (88 mg L⁻¹).

In all treatments, the levels of total ammonium nitrogen (TAN) increased from the beginning of the experiment, with maximum averages (14-17 mg L⁻¹) between days 14 and 21, subsequently decreased to 1 mg L⁻¹ at day 28, which was maintained until the end of the experiment (Fig. 1). The NO₂-N increased from day 14 to 28, later there was a slight decrease towards the end of the study. The NO₃-N levels increased from day 14 to the end of the study. The ANOVA test indicates no significant differences among treatments. The global means values of TAN varied from 5.5 mg L⁻¹ in (the control) to 6.5 mg L⁻¹ (treatment AS) (Table 2). The highest mean value of NO₂-N (17.1 mg L⁻¹) was recorded in treatment WB and the lowest (12.8) in OB. The highest mean value of NO₃-N was observed in treatment AS (28.8 mg L⁻¹), and the lowest in OB (23.8 mg L⁻¹).

Concerning the bioflocs proximate biochemical composition, no significant differences were found in organic matter or any of the nutrients among treatments (Table 3). The average of organic matter varied from 58.4% in the control to 62.5% in treatment WB. Protein content ranged from 33.4% in WB to 37.6% in OB. The lipid levels varied from 0.4% in the control to 0.5% in WB. The CHO content ranged from 17.1% in the control to 25.5% in WB.

The heterotrophic bacteria abundance associated to bioflocs when wheat bran was used as the substrate averaged 9.120×10⁶ CFU mL⁻¹, a value significantly higher than the 3.4×10⁶ CFU mL⁻¹ recorded in the control and the two other treatments (Fig. 2).

Some differences were observed in the production response of shrimp among treatments (Table 4). Survival ranged from 93.7% in the control to 98.8% in OB, but the differences were not significant. The final weight and the SGR were significantly higher in OB (1.38 g and 6.43% d⁻¹, respectively), as compared to the control (1.10 g and 5.83% d⁻¹). The FCR was significantly lower in OB (0.92) compared to the two other substrates (1.06), which still were lower than the control (1.20).

DISCUSSION

The water quality was not affected by the introduction of any of the three substrates considering that the main variables in all of the treatments and the control remained into the ranges considered as adequate for the intensive culture of shrimp. The tendency of the nitrogenous compounds (TAN, NO₂-N and NO₃-N) registered during the experiment was similar to previous

Table 1. Means and (ranges) of basic water quality variables recorded in the treatments and the control during the trial. DO: dissolved oxygen, TSS: total suspended solids, C: control, WB: wheat bran, AS: amaranth seeds, OB: oat bran. Different superscript in a column means significant differences at $P < 0.05$.

Treatment	Temperature (°C)	DO (mg L ⁻¹)	pH	TSS (mg L ⁻¹)
C	27.7 (26.5 - 28.8)	5.3 (4.7 - 6.2)	8.2 (7.6 - 8.8)	88 ^a (40 - 130)
WB	27.9 (26.4 - 28.6)	5.1 (4.5 - 5.5)	8.1 (7.5 - 8.7)	105 ^{ab} (40 - 170)
AS	28.0 (26.5 - 28.6)	5.0 (4.4 - 5.8)	8.1 (7.5 - 8.6)	130 ^b (50 - 200)
OB	27.9 (26.4 - 28.5)	5.0 (4.5 - 5.4)	8.2 (7.5 - 8.7)	115 ^{ab} (80 - 225)

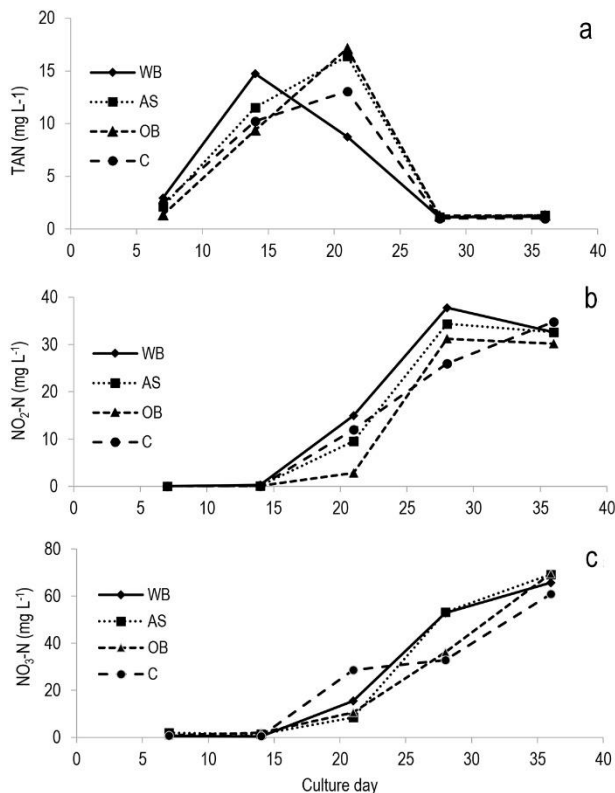


Figure 1. Mean values of a) total ammonia nitrogen, b) nitrite nitrogen, and c) nitrate-nitrogen (mg L⁻¹) during the experiment. WB: wheat bran, AS: amaranth seeds, OB: oat bran, C: control.

previous studies. Diverse authors have documented the initial increase of TAN during the first days of the culture and their consequent transformation to nitrite and nitrate; this coupled process is mediated by the ammonia oxidizers bacteria as well as the nitrite oxidizers (Esparza-Leal *et al.*, 2015; Moreno-Arias *et al.*, 2017; Zhang *et al.*, 2017; Rodrigues *et al.*, 2018).

The type of substrate added had a significant influence on the concentration of heterotrophic bacteria associated with the biofloc. The wheat brand was the most effective to be colonized by heterotrophic, reaching a concentration almost tripling that found on the control and the other two substrates.

The proximate biochemical composition of the biofloc was similar in all the treatments and the control. The amount of organic matter was slightly greater in WB, probably due to the higher concentration of bacteria, as has been reported by Azim & Little (2008). The protein content was slightly higher in OB, but this was expected to occur in the AS treatment, due to its higher protein content (65.6%) compared to wheat bran and oat bran (Chen *et al.*, 1988; Nascimento *et al.*, 2014). The lipid content of the biofloc was low in all treatments, ranging from 0.4 to 0.5%. Diverse studies have shown that lipid content may vary widely in the bioflocs, depending on many factors such as carbon source (Brito *et al.*, 2016) and the type of microbes associated (Martínez-Córdova *et al.*, 2015). Herein, the lipid contents in heterotrophic bacterial-based bioflocs are usually low (Xu & Pan, 2012; Long *et al.*, 2015), while these levels are higher in the photo-autotrophic biofloc (Becerra-Dorame *et al.*, 2012), mainly because of the contribution of diverse microalgae including diatoms, which additionally supply PUFA's and HUFA's (Martins *et al.*, 2016).

Some significant differences were observed in the production response of shrimp among treatments and the control, except for survival which was over 93% in all the studied groups. Similar high survivals were reported by Xu *et al.* (2018) during the intensive and hyper-intensive farming of *Penaeus (=Litopenaeus) vannamei* in a biofloc system. The final weight and the SGR were significantly higher in the treatment OB when compared to the control, while de FCR was the inverse, higher in the control than in OB. This pattern has been previously reported in many studies using bioflocs for the culture of fish and shrimp. For instance, Kim *et al.* (2014) found that *P. vannamei* postlarvae grew much more in a BFT than a traditional culture system.

Similarly, Emerenciano *et al.* (2012) reported a growth of 151 mg in clear water and 211 mg in a biofloc-based culture of *Farfantepenaeus brasiliensis* reared over 30 days, beginning from PL 25. The lower FCR obtained in treatment OB, suggests it could impro-

Table 2. Mean values (\pm SD) of nitrogenous compounds recorded in the treatments and the control during the trial. TAN: total ammonium nitrogen, C: control, WB: wheat bran, AS: amaranth seeds, OB: oat bran. In none of the columns were significant differences determined at $P < 0.05$.

Treatment	TAN (mg L ⁻¹)	NO ₂ -N (mg L ⁻¹)	NO ₃ -N (mg L ⁻¹)
C	5.55 \pm 5.69	14.58 \pm 15.55	24.72 \pm 25.20
WB	5.74 \pm 5.91	17.15 \pm 17.65	27.02 \pm 30.4
AS	6.50 \pm 7.04	15.35 \pm 17.03	28.85 \pm 31.99
OB	6.08 \pm 7.10	12.85 \pm 16.31	23.83 \pm 29.22

Table 3. Biochemical proximate composition (% of dry matter; mean \pm SD) of the bioflocs sampled in the treatments and the control at the end of the trial. OM: organic matter, CHO: carbohydrates, C: control, WB: wheat bran, AS: amaranth seeds, OB: oat bran. In none of the columns were significant differences determined at $P < 0.05$.

Treatment	OM (%)	Minerals	Lipids	Protein	CHO
C	58.37 \pm 1.87	41.63 \pm 1.87	0.408 \pm 0.162	36.42 \pm 7.53	17.14 \pm 3.80
WB	62.49 \pm 6.53	37.51 \pm 6.53	0.540 \pm 0.153	33.41 \pm 5.88	25.52 \pm 12.95
AS	60.67 \pm 5.88	39.33 \pm 5.88	0.495 \pm 0.298	35.34 \pm 8.28	21.83 \pm 11.55
OB	59.93 \pm 4.39	40.07 \pm 4.39	0.496 \pm 0.166	37.64 \pm 5.90	20.35 \pm 8.84

Table 4. Production parameters (mean \pm SD) of *Penaeus vannamei* reared in the treatments and the control. OM: organic matter, C: control, WB: wheat bran, AS: amaranth seeds, OB: oat bran. Different superscript in a column means significant differences at $P < 0.05$. SGR: specific growth rate, FCR: feed conversion ratio.

Treatment	Final weight (g)	SGR (% d ⁻¹)	Survival (%)	FCR
C	1.10 \pm 0.01 ^a	5.83 \pm 0.03 ^a	98.5 \pm 1.5	1.20 \pm 0.02 ^c
WB	1.28 \pm 0.15 ^{ab}	6.20 \pm 0.31 ^b	95.3 \pm 4.8	1.06 \pm 0.09 ^b
AS	1.29 \pm 0.10 ^b	6.24 \pm 0.20 ^b	93.7 \pm 6.0	1.06 \pm 0.08 ^b
OB	1.38 \pm 0.04 ^b	6.43 \pm 0.08 ^b	98.8 \pm 2.0	0.92 \pm 0.05 ^a

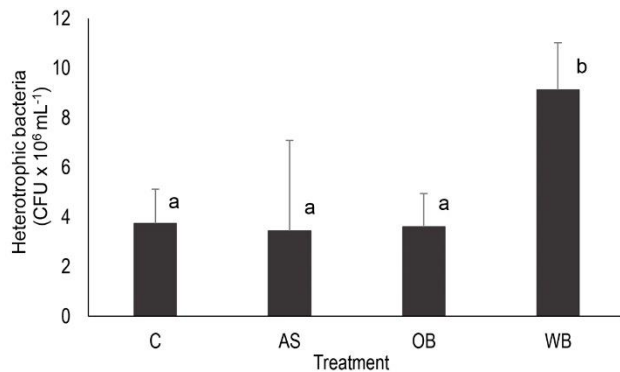


Figure 2. The abundance of heterotrophic bacteria associated with bioflocs in the treatments and the control. C: control, AS: amaranth seeds, OB: oat bran, WB: wheat bran. Different letter in the bars indicates significant differences at $P < 0.05$.

ve the feed digestion and assimilation. The FCR was also acceptable for the other two substrates and better than the obtained in the control. These results agree with those reported by Brito *et al.* (2016) who recorded

an FCR from 1.92 in the control to 0.92 in BFT farming of *P. vannamei*, and by Becerra-Dorame *et al.* (2012), in the pre-grown of the same species.

The improvement of the productive parameters registered in treatments with vegetable substrate addition may be induced by the selective colonization of beneficial bacteria. Some fibers have prebiotic effects, which promote selective stimulation of the growth of one or more bacteria, improving the nutrition and health of the host (Guergoletto *et al.*, 2010). In shrimp, future studies of proteomics could elucidate the metabolic pathways involved.

Finally, these results allow concluding that the addition of any of the three substrates showed no negative effects on the basic parameters of water quality, but some of them positively affected the production response of shrimp. The wheat bran resulted in being the most efficient substrate in terms of attachment of heterotrophic bacteria and organic matter, but the oat bran was the most effective to reduce the feed consumption, reflected in better growth and a lower FCR.

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