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Short Communication

Vitamin and mineral supplementation for *Macrobrachium rosenbergii* in biofloc system

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ABSTRACT. Biofloc system is an alternative to conventional aquaculture systems as it minimizes effluents, improves water quality, ensures greater biosafety due to minimal water exchanges and also serves as an additional food source for prawn. The present study aimed to evaluate the effect of suppression of vitamin and mineral supplements on diets for *Macrobrachium rosenbergii* reared in the biofloc system. Four experimental diets were evaluated: complete diet (Diet 1 with vitamin and mineral supplementation); diet without vitamin supplement (Diet 2); diet without mineral supplement (Diet 3) and diet without vitamin and mineral supplement (Diet 4). The experimental design was completely randomized with four replicates per treatment. After 45 days of trial survival rates above 90% and feed conversion rate ~1.83 were observed for all treatments, indicating that the production of *M. rosenbergii* in the biofloc system does not require the inclusion of vitamin and mineral supplementation in the feed.

Keywords: prawn farming, biofloc technology, food supplementation, diet, aquaculture.

Biofloc culture system, also known as Biofloc Technology (BFT), has shown positive results in terms of productivity and sustainability in prawn farming (Burford *et al*., 2004; Crab *et al*., 2010). BFT is considered an alternative to conventional systems by minimizing the effluents emission (Krummenauer *et al*., 2012), improving water quality (Crab *et al*., 2012) and ensuring greater biosafety in the rearing by minimize the introduction of diseases due to minimal water exchanges (Avnimelech, 1999). Bioflocs are constituted by organic particles suspended in the water column, is generally found in its composition heterotrophic bacteria, flagellates, ciliates, cyanobacteria, microalgae, nematodes and other small metazoans (Ballester *et al*., 2010).

The microorganisms present in the floc can convert nitrogenous compounds, such as ammonia, which is toxic to the reared organisms, into microbial protein (Avnimelech, 1999), serving as an excellent additional source of food for the prawn (Crab *et al*., 2010), besides to being a source of vitamins and minerals (Tacon *et al*., 2002; Ju *et al*., 2008).

Vitamins and minerals have broad participation in the metabolism of aquatic organisms; are required in small quantities for their healthy growth, reproduction, and health; and their supplementation must be adequate to the nutritional requirement of each species (Santos, 2007). Vitamins are organic compounds that serve as metabolic catalysts for various chemical reactions, that aquatic animals are not capable of synthesizing in sufficient amounts (NCR, 2011). Therefore, these must be supplemented on a diet (Gonçalves Jr. *et al*., 2015). As for the vitamins, minerals supplementation is also required on a diet to meet all macro and micromineral requirements necessary to ensure proper performance of prawn (NCR, 2011). Some minerals can be absorbed from water as calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe) and zinc (Zn) and others can be obtained through the natural productivity of nurseries, but generally does not meet the needs of the production organism (Shiau, 1998). According to Tacon *et al.* (2002) and Hargreaves (2013), microbial floc is a good source of vitamins and minerals and can supply the needs of these nutrients for shrimp. Therefore, the objective of the present study has evaluated the effect of suppression of vitamin and mineral supplements on diets for freshwater prawns *M. rosenbergii* reared in a biofloc system.

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The experiment was carried out at the Prawn Farming Laboratory of the Federal University of Paraná-UFPR-Palotina Sector, Paraná, Brazil, for 45 days. The experimental system, with water recirculation and biofloc formation, was installed in a closed room (14 m²) composed of 16 microcosms tanks (0.20 m², 50 L) and two macrocosms tanks (0.43 m², 310 L), with artificial substrates (plastic screens) installed in each tank (Schveitzer *et al*., 2013), constant aeration and no water exchange. Water circulation among the macrocosms and the microcosm tanks was continuous (1.2 L min^{-1} per tank). Halogen and fluorescent lamps were installed over the macrocosm tanks, with a photoperiod of 12 h light and 12 h dark (Araújo & Valenti, 2007). To stimulate the biofloc formation *M. rosenbergii* (50 prawns m^{-2}, initial weight of 11.94 ± 0.12 g) were stocked in the macrocosm tanks 30 days before the start of the experiment. In these tanks, ammonium chloride was added to increase the concentration of nitrogen compounds and to stimulate the development of microorganisms. The total ammonia nitrogen (N-NAT) level was limited to 1.0 mg L^{-1} using molasses (Avnimelech, 1999). Sodium bicarbonate (0.06 g L^{-1}) was added to maintain the water pH above 7.0 and dolomitic limestone (0.20 g L^{-1}) to increase the alkalinity above 100 mg L^{-1} (Furtado *et al*., 2011). Besides, 7.5 g of Sanolife MIC probiotic (Inve®) were added daily to assist in maintaining water quality. *M. rosenbergii* juveniles with an initial weight of 0.48 ± 0.11 g and initial length of 2.97 ± 0.09 cm, were used in the experiment (50 prawn m^{-2} in each microcosm). Four experimental diets were evaluated: complete diet (Diet 1 with vitamin and mineral supplementation); diet without vitamin supplement (Diet 2); diet without mineral supplement (Diet 3) and diet without vitamin and mineral supplement (Diet 4; Table1).

Prawns were fed three times a day (8:30, 13:30 and 18:30 h) with initial feeding rate equivalent to 10% of the biomass, adjusted according to observed consumption. The experimental diets were elaborated according to NRC (2011), to meet the nutritional requirement of the prawns (D'Abramo *et al*., 1997) and processed to obtain pellets with a 2.0 mm diameter. SuperCrac® software version 2.0 was used for calculations. The experimental diets were formulated to be isoproteic and isoenergetic (Table 1).

Temperature (digital thermometer-CE®), pH (pH meter AT-315-Alfakit®), dissolved oxygen (oximeter AT-170-Afakit®), conductivity (conductivimeter AT-230-Alfakit®) and turbidity (turbidimeter AP2000-PolyControl®) were monitored daily in the experimental units. Total ammonia and nitrite concentrations were analyzed daily, according to Mackereth *et al.* (1978), and the alkalinity and total hardness of the water were analyzed weekly, according to Walker (1978). The analyses were done in a spectrophotometer 2000UV (BE Photonics®). Floc volume was measured daily in ImHoff cone (Avnimelech, 2009).

At the end of the experiment, the animals were counted to evaluate survival (S). Prawns were measured with a digital caliper 402.150BL (King Tools®) and weighed in analytical scale AY 220 (Marte®) for evaluation of final weight, weight gain, apparent feed conversion rate, specific growth rate and total length. Results of the evaluated indices were submitted to analysis of variance (ANOVA) and the averages compared by the Tukey test ($P < 0.05$).

Water quality variables monitored during the experiment are presented in Table 2. The temperature registered was within the ideal range for the *M. rosenbergii* rearing, ranging from 24 to 31°C (New, 2002), and the pH suitable for the growth of aquatic organisms, between 6.5 and 8.5 (Boyd, 2000). In systems with limited water exchange, the alkalinity should be between 100 and 150 mg $CaCO_3$ L^{-1} to avoid fluctuations in pH (Ebeling *et al*., 2006). In the present study, the alkalinity was slightly below the recommended level but did not compromise the development of prawns. Floc volume was within the suitable values (Avnimelech, 2009) and the ammonia and nitrite levels remained stable in the rearing system, below 0.5 mg L^{-1}, as recommended by New (2002).

High survival rate at the end of the experiment (Table 3) indicates that the rearing system and the diets supplied were adequate for the development of prawns. Observed value, an average of 92.5% among experimental treatments, is satisfactory for prawn farming in a biofloc system. Pérez-Fuentes *et al*. (2013) observed survival rate of 85.4% for *M. rosenbergii* reared in a biofloc system. Presence of an artificial substrate in the rearing tanks also contributed to this result, as they improve the prawn growth and increase the survival rate by reducing the relative stocking density and relieving the stress of the animals (Schveitzer *et al*., 2013).

The microorganisms present in the biofloc provide essential nutrients for the development of prawns such as proteins, carbohydrates, lipids, vitamins and minerals (Tacon *et al*., 2002; Ju *et al*., 2008; Crab *et al*., 2010), favoring productive performance. Average feed conversion rate among the experimental treatments was 1.84 (Table 3), better than the value observed by El-Sherif & Mervat (2009) in a clear water system. These authors observed at the end of 45 days of rearing, average feed conversion rate of 2.35 for *M. rosenbergii* with initial weight and stocking density similar to use in the present study. In a biofloc system, the prawns have a better feed conversion rate compared

Table 1. The composition of the experimental diets. The experimental design was completely randomized with four replicates per treatment. Diet 1: with vitamin and mineral supplement, Diet 2: without vitamin supplement, Diet 3: without mineral supplement, Diet 4: without vitamin and mineral supplement. [1]Mix oil: 1:1 soy and fish oils. [2]Vitamin supplement (guarantee levels per kg): 16875 IU vitamin A, 3375 IU vitamin D_3, 200 IU vitamin E, 6.7 mg vitamin K_3, 120 mg vitamin B, 36 mg vitamin B_2, 25.5 mg vitamin B_6, 45 mcg vitamin B_{12}, 1200 mg vitamin C, 11.2 mg folic acid, 67.5 mg pantothenic acid, 170 mg nicotinic acid, 1.68 mg biotin, 265 mg inositol. [3]Mineral supplement (guarantee levels per kg): 65 mg iron, 13.8 mg copper, 150 mg zinc, 85 mg manganese, 0.35 mg cobalt, 1.3 mg iodine and 0.4 mg selenium. BHT: toluene butyrate hydroxide.

Ingredients	Diet			
	1	2	3	4
Diet ingredients (g kg^{-1} diet)				
Soy meal	350.00	350.00	350.00	350.00
Fish meal	256.50	256.50	256.50	256.50
Ground corn	200.00	200.00	200.00	200.00
Wheat bran	80.00	80.00	80.00	80.00
Starch	46.20	46.20	46.20	46.20
Mix oil[1]	41.90	41.90	41.90	41.90
Dicalcium phosphate	13.10	13.10	13.10	13.10
Vitamin supplement[2]	5.00	-	5.00	-
Mineral supplement[3]	5.00	5.00	-	-
B H T	0.20	0.20	0.20	0.20
Inert	-	5.00	5.00	10.00
Calculated nutrients				
Crude protein (%)	35.00	35.00	35.00	35.00
Ethereal extract (%)	7.20	7.20	7.20	7.20
Starch (%)	20.00	20.00	20.00	20.00
Gross energy (kcal kg^{-1})	4100	4100	4100	4100
Calcium (%)	1.87	1.87	1.70	1.70
Available phosphorus (%)	1.14	1.14	1.01	1.01

Table 2. Water quality variables monitored during the rearing of *Macrobrachium rosenbergii* juveniles for 45 days in a biofloc system.

Variable	Average ± SD
Temperature (ºC)	28.55 ± 1.05
pH	8.21 ± 0.40
Oxygen (mg L^{-1})	7.52 ± 0.63
Conductivity (µS cm^{-1})	0.77 ± 0.04
Turbidity (NTU)	3.30 ± 2.61
ammonia (mg L^{-1})	0.08 ± 0.07
Nitrite (mg L-^{1})	0.15 ± 0.06
Total hardness (mg $CaCO_3$ L^{-1})	97.04 ± 12.78
Alkalinity (mg $CaCO_3$ L^{-1})	94.95 ± 14.36
Floc volume (mL L^{-1})	1.00 ± 0.04

to traditional rearing systems (Pérez-Rostro *et al*., 2014), because crustaceans are animals that feed continuously, and the biofloc provides a rich source of natural food widely available throughout the rearing (Avnimelech, 1999; Pérez-Fuentes *et al*., 2013).

There was no difference (P > 0.05) among the experimental diets for all production indices evaluated (Table 3), all of which were satisfactory for the *M. rosenbergii* rearing. This result demonstrates that as the complete experimental diet, the diets without vitamin and mineral supplementation were well used by the prawns, and it was possible to conclude that, under the conditions of this study, the microbial flocs were able to supply the needs of vitamins and minerals for the prawns. Calcium, magnesium, phosphorus, sodium, iron, zinc, potassium and trace of others minerals can be found in the microbial aggregates (Tacon *et al*., 2002), as well as vitamins A, D, E, K (Ju *et al*., 2008) and vitamin C (Crab *et al*., 2010). Similar results were noted by Decamp *et al*. (2002). They observed that it is possible to reduce or remove vitamin and mineral supplements from the diet without affecting the growth and survival of *L. vannamei* reared in nursery conditions (green water), and the same result was observed by Velasco & Lawrence (2000) with a diet without vitamin supplementation for *L. vannamei* reared in a system without water exchange. In a study with *M. rosenbergii*, Tidwell *et al*. (1995) suggest that prawns can increase the level of natural biota predation to supplement their nutritional intake when essential nutrients are deleted from the diet.

Table 3. Average values ± (SD) of survival (S), final weight (FW), weight gain (WG), total length (TL), feed conversion rate (FCR) and specific growth rate (SGR) of *Macrobrachium rosenbergii* juveniles reared for 45 days in a biofloc system and fed diets with and without vitamin and mineral supplementation. Diet 1: with vitamin and mineral supplement, Diet 2: without vitamin supplement, Diet 3: without mineral supplement, Diet 4: without vitamin and mineral supplement.

Variable	Diet			
	1	2	3	4
S (%)	95.00 ± 10.00	90.00 ± 14.44	90.00 ± 11.54	95.00 ± 10.00
FW (g)	2.06 ± 0.13	2.19 ± 0.12	2.15 ± 0.16	2.18 ± 0.16
WG (g)	1.64 ± 0.26	1.77 ± 0.24	1.73 ± 0.32	1.75 ± 0.33
TL (mm)	59.0 ± 1.93	60.95 ± 1.77	67.65 ± 1.00	58.98 ± 2.90
FCR (g/g)	1.87 ± 0.28	1.83 ± 0.38	1.93 ± 0.44	1.73 ± 0.48
SGR (% day^{-1})	3.48 ± 0.28	3.62 ± 0.24	3.57 ± 0.33	3.60 ± 0.34

In conclusion, this study shows that in BFT systems for prawn production there is no need for supplementation of vitamin and mineral in the diets.

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