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Giant bladder kelp (*Macrocystis pyrifera*) and maize (*Zea mays*) meals as nucleation sites for biofloc formation

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ABSTRACT

The aim of this study was to evaluate *Macrocystis pyrifera* and *Zea mays* meals as nucleation sites in biofloc formation. Bioflocs were studied for a period of 12 weeks, by measuring volume, dry weight, organic matter, and ash weight. These were induced by the addition of bacterial inoculum, molasses and both types of meals to fresh water. After 4 weeks, a total of 100 tilapia fingerlings (1.60 ± 0.02 g weight and 4.76 ± 0.03 cm length) were transferred to 100 L tanks by triplicate for each treatment. Mean ± SD values of temperature (26.3 ± 0.2 °C), dissolved oxygen (3.42 ± .0.07 mg L^{-1}), pH (7.95 ± 0.04), salinity (0.47 ± 0.01) and ammonia (0.245 ± 0.012 mg NH_3 L^{-1}) were similar in both treatments. However, floc volume of *M. pyrifera* was significantly higher (116.1 ± 20 ml L^{-1}) than *Z. mays* flocs (58.4 ± 7.7 ml L^{-1}), but had lower dry weight (55 ± 0.5 mg g^{-1} in *M. pyrifera* vs. 61 ± 2.0 mg g^{-1} in *Z. mays*). Fish weight and length gain and survival were significantly higher ($p < 0.05$) in *M. pyrifera* floc (13.7 ± 1.3 g; 4.5 cm; 90 %) than in *Z. mays* (12.3 g; 8.5 cm; 56 %). Aggregated organisms were classified into major groups and were quantified and identified to a genus level. Ciliates were detected on week 1 and rotifers and nematodes were first registered on weeks 3 and 4. These three groups were present in both treatments but were more abundant in maize biofloc. In conclusion, both types of nuclei resulted in the formation of bioflocs rich in aggregated organisms, but nuclei of *M. pyrifera* produced a significantly higher performance of tilapia fingerlings, probably because of the higher nutritional content of kelp over maize meal and higher flotation of *M. pyrifera* flocs, which remained longer in suspension making them readily available to the fish.

1. Introduction

Biofloc technology (BFT) has emerged as an eco-friendly and innovative technique to increase efficiency, recycle nutrients, lower water usage and running costs of aquaculture systems, while reducing the adverse effects inflicted on the environment (Avnimelech, 2015; Azim and Little, 2008; Luo et al., 2014). The system is based on the knowledge of conventional domestic wastewater treatment systems and applied in aquaculture environments. Nutrients generated by the system are recycled and continuously reused by communities of organisms present. However, for floc formation, biological polymeric substances are required, which have the function of binding and holding the components together, forming a matrix that aggregates the microorganisms (bacteria, protozoa and fungi) facilitating their ingestion by fish, providing direct access to nutrients and acting as a substrate (De Schryver et al., 2008). Each floc remains attached by a matrix of mucus secreted by bacteria, filamentous microorganisms or by electrostatic attraction.

To shorten the start-up period in using BFT, Crab et al. (2012) and Ahmad et al. (2017) have called for the need to investigate the effect of adding nucleation sites to the water at start-up, which will stimulate floc formation (Gaona et al., 2011). The start-up period could also be shortened by inoculating the water with existing good-performing biofloc or with specific inoculum. Corn and wheat meals are commonly used to induce a higher floc formation because of its starch content, which is a natural coagulant and favors creation of microbial

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communities around them. Martínez-Córdova et al. (2018) compared amaranth and wheat grains as nucleation sites of microbial communities to produce bioflocs used for shrimp culture. Macroalgae have not been tested as nucleation sites in floc formation, regardless they have high protein and low carbohydrates contents and some other properties, such as inhibitors of undesirable bacterial growth of various pathogens because of the presence of phenolic, terpenoid and lipophilic compounds responsible for antimicrobial activity (Chiheb et al., 2009; Gupta and Abu-Ghannam, 2011). They could also serve as good nucleus because of their high content of alginates used as thickener and gelling agent.

The giant bladder kelp *Macrocystis pyrifera* is a brown alga underexploited in the northern west coast of Mexico, from which alginates are extracted and liquid fertilizers are obtained for agricultural purposes (DOF, 2012). There is a potential exploitable biomass of 80,000 t/year of this species from which dry meal could be produced for nucleation sites of biofloc, without competing with other grains of meals used for human consumption such as wheat, corn and other cereals. The aim of this work was to evaluate two meals (kelp and maize) as nucleation sites of biofloc formation and test their effect on performance of *Oreochromis niloticus* fingerlings.

2. Materials and methods

2.1. Experimental conditions

The experiment was performed during 12 months in indoor plastic-tanks of 150 L, situated in the Laboratory of Research, Technological Development and Innovation in Tilapia, Nayarit Unit of Centro de Investigaciones Biológicas del Noroeste (UNCIBNOR), in Tepic Nayarit, México. Meals of *Macrocystis pyrifera* and *Zea mays* of different proximal composition (Table 1) were evaluated as nucleation sites of biofloc formation and later, we tested their effect on performance of *O. niloticus* fingerlings. The kelp meal was provided by Algas Pacific® Enterprise and maize meals were bought locally. Both meals were sieved to 400-μm particle size.

2.2. Biofloc development and assessment

The six plastic tanks of 150 L were filled with 100 L of tap water, which were aerated for 24 h for chlorine elimination. A total of 5 L of a bacterial inoculum and molasses were added to each tank and were left for 24 h before nucleation site meals were added. The bacterial inoculum was prepared, with 1.37 g of Epicin-hatcheries® containing *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans, Lactobacillus acidophilus* y *Saccharomyces cerevisiae*, and 0.68 g molasses dissolved in 60 L of fresh water with aeration for 24 h in a separated tank. One day later, 1.27 g of *Macrocystis pyrifera* and *Zea mays* meals were added daily to each of the three tanks respectively, as well as molasses (sugar cane) and commercial feed (Purina, Nutripec®, protein 40 %, lipid 15 % and ash 15 %) to maintain a C:N ratio of 15:1, according to Avnimelech et al. (1994). Each tank was agitated and aerated continuously using a blower (S-65-3, 4.8 HP) to ensure that dissolved oxygen remained at saturation level; pH, temperature, salinity, and total ammonia nitrogen (TAN) were measured twice daily, at 10:00 and 15:00 h, using a multiparameter meter YSI Environmental 556 (Yellow Springs Incorporated®) and an API Ammonium Test Kit (Aquarium Pharmaceuticals®), respectively. Non-ionized fraction of TAN (ammonia) was calculated based on temperature and pH values (Emerson et al., 1975).

Floc volume was determined by sampling 1 L of water contained in each tank, which were set into a series of Imhoff cones. After 20 min, the volume of floc plug accumulated on the bottom of the cone was determined. Thus, total volume was homogenized, and three samples of 1 mL was taken from each tank (18 in total) and preserved with a buffered formalin solution (4 %) (Thompson et al., 2002). Microorganisms of the preserved samples were classified into major groups (microalgae, ciliates, annelids, gastrotrichia and rotifers) and were identified at genus level. Ciliates, annelids and rotifers were counted using a microscope (10x, Optika Microscope®).

Table 1
Proximal composition of kelp (*Macrocystis pyrifera*) and maize (*Zea mays*) meals used as nucleation sites for biofloc development.

Component	*Macrocystis pyrifera**	*Zea mays***
	(%)	(%)
Protein	9.41	7.35
Lipids	0.85	1.53
Carbohydrates	39.59	81.40
Humidity	19.15	9.29
Ash	31.00	0.43

Figures are expressed in wt % dry basis. * Castro-González et al. (1994).** Toro et al. (2011).

2.3. Nile tilapia production test

After 4 weeks of biofloc induction, 100 fingerlings of *O. niloticus* (1.60 ± 0.02 g and 4.76 ± 0.03 cm) were introduced in each tank at a density of 1,000 in.. m^{-3}. Fish were fed 1 mm pelleted commercial feed (Nutripec®, Purina, tilapia starter, protein 40 %, lipid 15 % and ash 15 %) twice a day at 10:00 and 15:00 h at a daily rate of 5 %; feeding were adjusted daily in relation to biomass in each tank. A carbohydrate source in form of molasses was added, as suggested by Hargreaves (2013) and calculated using the following equation developed by Avnimelech (2015): ΔCH = ΔFeed x %N in feed x %N excretion / 0.05. Both *Macrocystis pyrifera* and *Zea mays* meals were added daily in relation to the desired biofloc volume of 50 ml L^{-1}. Fermentable sugar content of molasses was 46–49 % W/W. Every week, 20 fish in each tank were sampled and length and weight were measured with an ichtyometer (cm) and a digital scale Scout pro OHAUS® (0.01 g precision), respectively. The specimens were also examined to detect any skin or fin damage. Dead fish were removed from the tanks. At the end of the study, all fish of each tank were harvested for total biomass determination.

To evaluate the effect of kelp and maize bioflocs, the following production parameters were estimated: Weight gain WG (g) = Final weight – initial weight; Average daily weight gain (ADG) (g day^{-1}) = final weight – initial weight/days; Length gain (LG) (cm) = Final length – initial length, Average daily length gain (ADL) (cm day^{-1}) = final length – initial length/days; Apparent food consumption (AFC) (g ind^{-1}) = Food given/number of fish; Feed conversion ratio (FCR) = Food given/weight gain; Protein efficiency ratio (PER) = Weight gain/protein apparently consumed.

2.4. Statistical analysis

Data are presented as means ± standard error. Significant differences between treatments (*Macrocystis pyrifera* and *Zea mays*) and time (weeks) were assessed by two-way ANOVA followed by the Tukey HSD post hoc test for mean comparisons at $P < 0.05$. Data were previously subjected to normality (Kolmogorov-Smirnov, $\alpha = 0.05$) and homocedasticity (Bartlett, $\alpha = 0.05$) tests. Percentage data were arcsine square root transformed (Zar, 1984) before analyses. All statistical tests were performed using Statistica 8.0 software.

3. Results

3.1. Water quality

Mean water quality parameters of both biofloc systems did not differ statistically (Table 2). Mean temperature of all tanks throughout the experiment was 25.16 ± 0.16 °C in the morning and 27.53 ± 0.14 °C

Table 2
Water quality parameters in the tanks during 12 weeks in the two biofloc systems.

Parameter	Unit	*Macrocystis pyrifera*	*Zea mays*
Temperature (10:00 h)	°C	25.06 ± 0.18	25.06 ± 0.18
Temperature (15:00 h)	°C	27.53 ± 0.14	27.53 ± 0.14
Dissolved Oxygen (10:00 h)	mg L^{-1}	3.54 ± 0.05	3.54 ± 0.05
Dissolved Oxygen (15:00 h)	mg L^{-1}	3.31 ± 0.09	3.31 ± 0.09
Salinity (10:00 h)	ups	0.41 ± 0.01	0.41 ± 0.01
Salinity (15:00 h)	ups	0.53 ± 0.01	0.53 ± 0.01
pH (10:00 h)	–	8.03 ± 0.02	8.03 ± 0.02
pH (15:00 h)	–	7.87 ± 0.06	7.87 ± 0.06
Ammonium (NH_4^-)	mg L^{-1}	4.1 ± 0.2	3.6 ± 0.2
Ammonia (NH_3)	mg L^{-1}	0.261 ± 0.012	0.230 ± 0.012
Floc volume	ml L^{-1}	116.1 ± 20^b	58.4 ± 7.7^a
Floc dry weight	mg ml^{-1}	55 ± 0.5^a	61 ± 2.0^b
Floc ash weight	mg ml^1	41.5 ± 0.5	47 ± 1.5
Floc organic matter	mg ml^{-1}	13.5 ± 1.0	14.5 ± 3.0

Figures are the mean ± standard error. Different superscripts between treatments per parameter show statistical differences ($p < 0.05$).

in the afternoon. DO had a mean of 3.54 ± 0.05 mg O_2 L^{-1}, pH averaged 8.03 ± 0.02 and mean salinity was 0.53 ± 0.02. The mean values for TAN was 4.1 ± 0.2 mg L^{-1} and 3.6 ± 0.2 mg L^{-1} for *M. pyrifera* and *Z. mays*, and calculated non ionized ammonia were 0.261 ± 0.012 and 0.23 ± 0.012 mg $L^{-1,}$ respectively. Biofloc volume of *M. pyrifera* was significantly higher (116.1 ± 20 ml L^{-1}) than that for *Z. mays* (58.4 ± 7.7 ml L^{-1}) (Table 2). Nevertheless, floc dry weight of kelp meal (55 ± 0.5 mg g^{-1}) was significantly lower than maize floc (61.20 ± 2.0 mg g^{-1}). This indicates a lower density and higher flotability of *M. pyrifera* flocs, making them available for fish for longer periods as seen during the 20 min tests with Imhof cones.

3.2. Biota assessment

Abundant bacteria and microalgae developed in both treatments after inoculation. These were not quantified but a higher abundance of microalgae was observed in kelp biofloc. Four genus of Chlorophyta one of diatom, eleven genus of ciliates, two annelids, one gastrotrichia and seven rotifers were identified (Table 3). Fig. 1 shows the relative abundance of major microorganism groups in flocs of both treatments during the 12 experimental weeks. Ciliates were found during the first week onwards followed by rotifers on week 3 and finally annelids on week 4. Ciliates were most abundant than rotifers and annelids in both treatments. Relative abundance (individuals) of these groups was significantly lower in kelp than in maize flocs (ciliates 86 ± 15 vs. 233 ± 25; rotifers 21 ± 7 vs. 73 ± 12; annelids 9 ± 2 vs. 37 ± 5).

3.3. Productive performance of tilapia

Initial culture Nile tilapia conditions were similar in density, weight and size of fish in both treatments (Table 4). At the end of the experiment, significant differences were found in survival. In *M. pyrifera* floc, mean survival of fingerlings was 90 % compared with 56 % obtained in *Z. mays floc* (Fig. 2). Dead fish were not replaced and therefore density dropped from 1,000–900 and 560 fish m^{-3} in kelp and maize flocs respectively. Nonetheless, fish in kelp biofloc showed a significantly higher weight and length gain (Fig. 2). Average daily weight and length gains, protein efficiency ratio and Feed Conversion ratio were also significantly ($p < 0.05$) better in *M. pyrifera* (Table 4). Apparent food consumption was not significantly different in both treatments, but biomass harvested from all tanks were 3318 g in *M. pyrifera* vs. 1326 g in *Z. mays* bioflocs respectively.

Table 3
List of microbiota found in bioflocs nucleated with *Macrocystis pyrifera* and *Zea mays* meals during 12 weeks of study.

Genera	Taxa	*Macrocystis pyrifera*	*Zea mays*
Microalgae			
Ankistrodesmus sp.	Chlorophyta	X	X
Coelastrum sp.	Chlorophyta	X	X
Monoraphidium sp.	Chlorophyta	X	–
Navicula sp.	Diatom	X	X
Scenedesmus sp.	Chlorophyta	X	X
Ciliates			
Askenasia sp.	Ciliophora	X	X
Aspidisca sp.	Ciliophora	X	X
Chilodonella sp.	Ciliophora	X	X
Coleps sp.	Ciliophora	X	X
Epistylis sp.	Ciliophora	X	X
Litonotus sp.	Ciliophora	X	X
Paramecium sp.	Ciliophora	X	X
Podophrya sp.	Ciliophora	–	X
Stylonychia sp.	Ciliophora	X	X
Tokophrya sp.	Ciliophora	–	X
Vorticella sp.	Ciliophora	X	X
Annelids			
Aelosoma sp.	Annelida	X	X
Tubifex sp.	Annelida	X	–
Gastrotrichia			
Chaetonotus sp.	Gastrotrichia	–	X
Rotifers			
Cephalodella sp.	Rotifera	X	X
Colurella sp.	Rotifera	X	–
Epiphanes sp.	Rotifera	–	X
Lecane sp.	Rotifera	X	X
Lepadella sp.	Rotifera	X	X
Philodina sp.	Rotifera	X	X
Proales sp.	Rotifera	X	X

4. Discussion

The environmental conditions at which the experiment was performed were similar between treatments and their mean values were within acceptable values recommended by FAO (2019) for tilapia aquaculture. Water temperature averaged 26 °C, which was within the temperature range for tilapia development and growth that is 20 – 35 °C (El-Sayed, 2006) and not far from optimum range for growth that is 29 – 31 °C (Popma and Lovshin, 1996). Dissolved oxygen concentration remained above 3.54 ± 0.05 mg L^{-1}, which is the lowest level recommended by Boyd and Tucker (1998) for tilapia aquaculture. At this concentration, food intake and fish growth rate is not affected. In addition, O_2 levels were adequate for growth of heterotrophic and nitrifying bacteria (Timmons and Ebeling, 2010).

There were no significant differences between ammonium (NH_4) concentration in both treatments and the mean value (3.85 mg L^{-1}) was similar to those reported by Wambach (2013) in *O. niloticus* cultivated in BFT that were 3.32–4.48 mg L^{-1}. As temperature and pH were also similar, non-ionized ammonia (NH_3-) concentration were similar in both treatments with a mean value of 0.245 mg L^{-1}. This value is far from the LC_{50} 96 h (1.83 mg L^{-1}) determined by Bravo-Yumi (2007) for *Oreochromis* sp., despite no water exchange was made during the duration of the experiment. Indeed, this is one of the most important advantages of BFT and is the result of bacteria fed with carbonaceous substrates (molasses), to take up nitrogen from the water for protein production (Avnimelech, 2015). Tilapia production in BFT may be 43 % higher than the production in tanks without biofloc (Muñoz Kuehne, 2018).

Particulated organic matter as well as other organisms in the microbial food web, have been proposed as potential food sources for aquatic animals (Baylor and Sutcliffe, 1963; Martínez-Córdova et al., 2017; Moriarty, 1997). Microorganisms in floc-based systems are thought to have an important role in cultured animal nutrition. In the

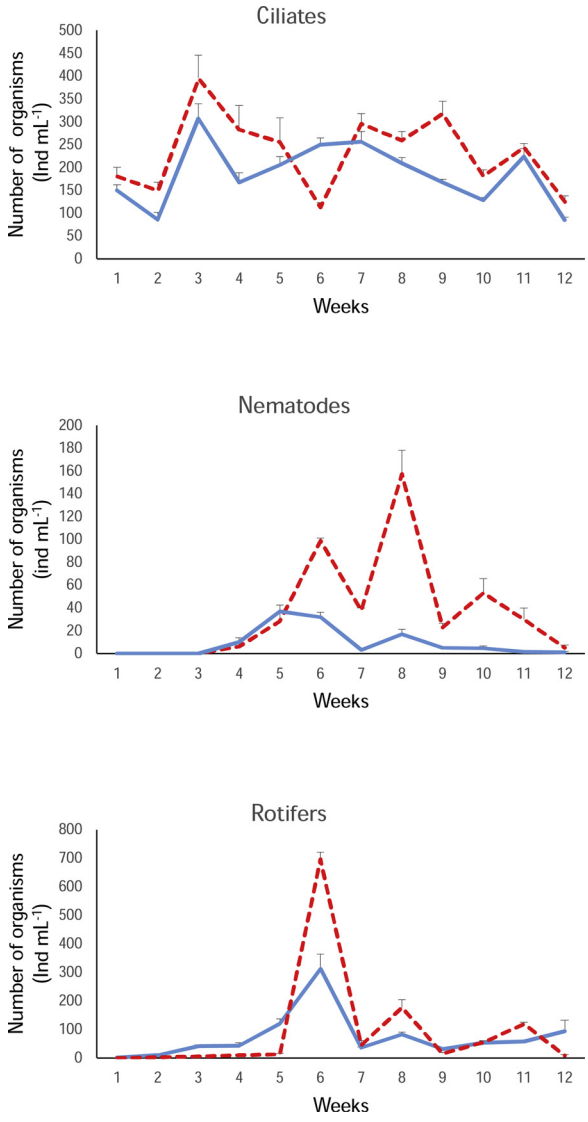

Fig. 1. Variation of relative abundance of major groups of microorganisms found in flocs nucleated with *Macrocystis pyrifera* (solid lines) and *Zea mays* (broken lines) meals during the 12-week experiment. Values are the mean ± standard error of the mean (n = 18).

present work both meals proved to be excellent nucleation sites in biofloc formation. In only few days, nucleation sites were colonized by microalgae (mainly Chlorophytes), ciliates and rotifers. According to Avnimelech (1999; 2007), these microorganisms provide a good source of nutrients to fish, which is available 24 h a day. In the case of rotifers, they can fragment floccules and consume present bacteria, processing and transferring the energy generated to the biofloc, as well as helping to promote the generation of more floccules because of their contribution of mucilage produced by their excreta (Pérez, 2014).

We ignore the origin of such organisms especially during the first three weeks from inoculation. However it is possible that annelids and gastrotrichia were introduced with the fingerlings because these were detected just after week 4.

Maize floc had a significantly higher relative abundance of ciliates, annelids and rotifers and it was heavier than the kelp floc. Therefore, we would expect a better performance of fish in this floc. However, results indicate the opposite; weight and length gain, and survival were significantly higher in kelp biofloc, even considering that fish in maize floc showed mortality and therefore raised at a significant lower density from week 7 onwards.

Table 4
Performance parameters of tilapia *Oreochromis niloticus* fingerlings cultured in biofloc nucleated with *Macrocystis pyrifera* and *Zea mays* meals during an 8-week experiment.

Parameter	*Macrocystis pyrifera*	*Zea mays*
Initial density (ind m^{-3})	1000	1000
Final density (ind m^{-3})	900 ± 75.5 [a]	560 ± 151.0 [b]
Survival (%)	90.0 ± 7.6 [a]	56.0 ± 15.1 [b]
Initial weight (g)	1.69 ± 0.0	1.57 ± 0.0
Final weight (g)	15.36 ± 1.4 [a]	12.30 ± 1.8 [b]
Weight gain (g)	13.67 ± 1.3 [a]	10.73 ± 1.8 [b]
Average daily weight gain (g day^{-1})	0.24 ± 0.0 [a]	0.19 ± 0.0 [b]
Initial length (cm)	4.8 ± 0.0	4.68 ± 0.0
Final length (cm)	9.3 ± 0.1 [a]	8.5 ± 0.4 [b]
Length gain (cm)	4.5 ± 0.1 [a]	3.8 ± 0.3 [b]
Average daily length gain (cm day^{-1})	0.08 ± 0.0 [a]	0.07 ± 0.01 [b]
Total food consumption (g)	980.7 ± 21.3 [a]	638.5 ± 98.9 [b]
Apparent food consumption (g $food^{-1}$ day^{-1})	0.19 ± 0.01	0.17 ± 0.01
Feed conversion ratio	1.16 ± 0.14 [a]	1.32 ± 0.34 [b]
Protein efficiency ratio	0.98 ± 0.14 [a]	0.76 ± 0.15 [b]
Total biomass harvested (g) †	3318	1326

Figures are the mean ± standard error of the mean. Subscript letters within rows indicate significant differences between treatments ($p < 0.05$). (†) No statistical analysis was performed.

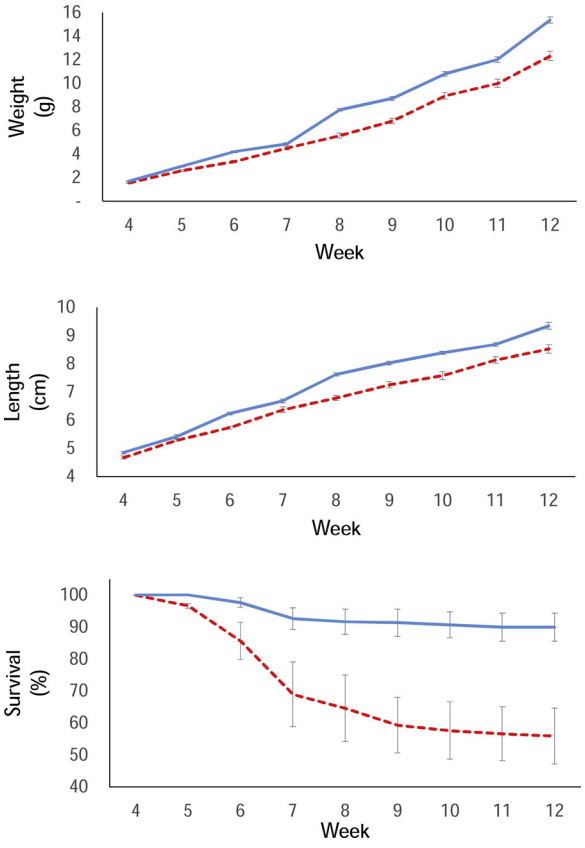

Fig. 2. Growth in weight (g) and size (cm), and survival (%) of *Oreochromis niloticus* fingerlings, grown in bioflocs nucleated with *Macrocystis pyrifera* (solid lines) and *Zea mays* (broken lines) meals during 8 weeks of experiment. Values are the mean ± standard error of the mean (n = 60).

Two main factors may be the cause of this result. Firstly, the particles of *M. pyrifera* floc have apparently higher floatability and as a consequence remained longer in suspension and readily available for fish feeding (Martínez-Córdova et al., 2018). Floc of *M. pyrifera* formed a larger plug of spongy consistency in Imhoff cones, and also the particles remained longer in suspension even after the 20 min

sedimentation test. The spongy consistency may be due to the high alginate content, which is a thickener and gelling agent (Cervantes-Cisneros et al., 2015). In contrast, maize floc particles were more compact and formed a smaller plug of 58.4 ml L^{-1} in the same period of time. This is in agreement with Hargreaves (2006), who states that the nutritional value of biofloc to aquatic animals is not dependent only on the ability of fish to both ingest and digest the particles, but also on the density of suspended particles.

The other factor could be due to differences in the proximal composition of meals. Kelp meal has a higher protein content (9.41 %) than maiz meal (7.35 %) but lower content of lipids and carbohydrates (Table 1). It is known that early juvenile fish (0.02–10.0 g) would require a diet higher in protein, lipids, vitamins and minerals and lower in carbohydrates (FAO, 2019). This could explain the mortalities registered in maize treatment starting in the 7th week of the experiment. Similar results are reported by Martínez-Córdova et al. (2018) who found that amaranth grain with the highest protein content tested as nucleation sites produced higher growth of shrimp. In addition, *M. pyrifera* as substrate in a biofloc system has shown compounds that inhibited undesirable bacterial growth including pathogens. These are phenolic, terpenoid and lipophilic compounds that are responsible for antimicrobial activity (Chakraborty et al., 2010; Chiheb et al., 2009; Manilal et al., 2012). Bacterial macroalgal inhibition properties could be favoring a decrease in pathogen bacteria and promoting denitrifying bacterial establishment such as *Nitrospira* sp., *Nitrobacter* sp. and *Bacilus* sp. (Avnimelech, 1999; Avnimelech et al., 2009; Crab et al., 2012; Hargreaves, 2006). Regardless the superiority of *M. pyrifera* in relation to *Z. mays* bioflocs in *O. niloticus* fingerlings, a similar study is required at later stages of development, since it is known that sub-adult fish ($10-25$ g), require more energy from lipids and carbohydrates for metabolism and a lower proportion of protein for growth (FAO, 2019). Adult fish (>25 g) would require even less dietary protein for growth and can utilize even higher levels of carbohydrates as a source of energy (FAO, 2019).

The meal of *M. pyrifera* in Mexico cost approximately US$ 1.00/kg, which is 28 % more expensive than maize meal that cost approximately US$ 0.73. The impact of the difference on a real operation would be negligible since nucleation meal represents only 0.26 % of total production costs with BFT in a commercial farm (Espinosa-Chaurand et al., 2019). Subsequently, it is likely that the use of *M. pyrifera* meal is cost effective taking into account that total biomass harvested. In our study, the biomass produced was 150 % higher using *M. pyrifera* floc than using *Z. mays* floc.

5. Conclusion

Both types of meals tested served as nucleation sites for biofloc formation. *Macrocystis pyrifera* meal produced higher performance of tilapia fingerlings than maize meal, probably because of a higher nutritional (protein) content and higher flotation of flocs which make them readily available to the fish. No restriction is foreseen in considering *M. pyrifera* meal in biofloc development because there are unexploited reserves of this algal species in northern west coast of Mexico. The use of kelp meal reduces the demand for cereals destined for human consumption.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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