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Effect on nutritional composition of produced bioflocs with different carbon sources (Molasses, coffee waste and rice bran) in Biofloc system

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Abstract
The mean goal of this study was to evaluate the effect of rice bran, molasses, and coffee waste in nutritional composition of floccules, and associated micro fauna. Each treatment was made by triplicate in 80L plastic containers with 15 tilapias each one. Parameters like temperature, pH, dissolved oxygen, NO_2-, NO_3-, and TAN were monitored. Associated micro fauna was estimated by direct count method and proximal analysis was made by standardized methods of AOAC. The results did not show significant differences with respect water quality during all experiment. The associated micro fauna was represented by microalgae, protozoa, and rotifers. Proximal analysis did not show significant differences with respect crude lipids, with relation crude fiber, coffee waste treatment obtained highest percentage (29%), for ashes, molasses obtained the highest value (16.46%), rice bran treatments and molasses showed high protein levels (47,99 and 42.01% respectively). The results indicate that regardless of the carbon source used, the Biofloc contributes as a source of food *in situ*, which can be comparable with conventional food but without the economic impacts that this generate.

Keywords: carbon source, phytoplankton, zooplankton, biofloc, aquaculture nutrition

1. Introduction
In Aquaculture production systems, feeding represents the biggest expend to producers, because it can be 40% of operating costs, besides that a high percentage of supplied food is not consumed by organisms [1]. Biofloc system is known like good culture alternative to decrease feeding costs, because is based on generating microbial floccules as *in situ* microbial protein source [2] and amino acids [3] that can be used as primordial feeding source of cultured species [4, 5, 6]. Carbon source is the main input to good management of Biofloc system, so choice of this source can cause variations in nutritional value and associated micro fauna [7]. In a study using molasses as carbon source [8], 28.7 to 43.1% of protein and 2.11 and 3.625 of lipids were reported in bioflocs used to cultured *Litopenaeus vannamei.* In other study cultured tilapia with wheat flour [9], was obtained protein levels of 38%, and for lipids of 3.16 and 3.23%, likewise [10], was made a tilapia culture in closed system, substituting partially formulated diet with bioflocs formulated with molasses, and was observed that compositions of bioflocs show optimal levels of proteins and lipids.

Bioflocs are partly formed with a wide microorganism's diversity like: bacteria, microalgae, yeast, rotifers, ciliates, protozoa, nematodes, and copepods [11, 12]. These microorganisms are used in aquaculture, taking in consideration their nutritional contribution and their ecological importance, because are the basis of all aquatic food chain [13]. For example, microalgae protein content may vary to 30 to 65% of dry weight [14]. In chlorophytes and diatoms, saturated fatty acids can constitute 15 to 40% of total fatty acids, meanwhile green microalgae show low concentrations of monounsaturated fatty acids and high polyunsaturated concentrations [15]. Proximal composition of some planktonic species that are found in bioflocs show that rotifers can contain 54 to 60% of crude protein, whereas cladocerans 50 to 68%, and copepods 70 to 71% [16, 17].

In recent years, were published different studies about positive effects in fish and crustacean's growth cultured in Biofloc, however, was only few reports about nutritional content of bioflocs that were development in this system and their relationships with carbon source applied. For the above, it is necessary extend the studies that allow evaluation of carbon source applied can modified organism's composition associated to Biofloc system and bioflocs nutritional content.

2. Materials and methods
2.1 Experimental design
Outside plastic containers with 80 L capacity were used to cultured organisms, filled with dechlorinated water, and 15 tilapias of (*Oreochromis niloticus*) each one with mean weight of 4.2 ± 1.08 g. Daily tilapias were fed with commercial diet (ALIMENTOS DEL PEDREGAL®, Toluca, Estado de México, México), with 35% content of protein and particle size of 0.6-0.8 mm.

During experiment, no water change was made, but evaporation water lost was compensated with freshwater dechlorinated. To promote bioflocs generation was used three carbon sources: molasses (MOL), rice bran (RB) and coffee waste (CW), and control (without carbon source). Each treatment was made by triplicate and was assigned aleatory. Carbon source and food were applied once a day with a C/N ratio of 20:1. The daily amount of added carbon was calculated according to Avnimelec [18]. Container water was aerated and stirred with air stone diffusers connected to air pump. Aeration tube diameter was 6 mm, and air stone diameter was 40 mm. The experiment was carried out over a period of 12 weeks.

2.2 Water quality parameters evaluation.
Once a week, water quality parameters were done. Water temperature (°C), dissolved oxygen, and pH were determined by multiparametric HANNA equipment, HI9829 Model (Woonsocket, Rhode Island, USA). Total ammoniacal nitrogen levels (TAN, mg/L), nitrites (NO_2^- mg/L), nitrates (NO_3^- mg/L) were made by spectrophotometry using HANNA autoanalyzer Aquaculture Photometer model HI83203 (Woonsocket, Rhode Island, USA), according with standard methods of manufacturer.

2.3 Planktonic characterization
Every week, bioflocs were concentrated to identify microorganism, using a mesh screen of 10 µm, and diluted in 100 mL of distilled water. Later, 150 µL sample were taken and fixed with 5% formalin solution, to observe and directly counting on standard frosted slides 76x26 mm with thickness of 2 mm, making four sweeps over the slide using optical microscope (OLYMPUS BX50, Tokyo, Japan), with phases contrast. Different observed group identified was made according with taxonomic keys of Aladro [19] and Patterson [20].

2.4 Bioflocs proximal analysis
Bioflocs proximal analysis were concentrated samples from each culture container through 10 µm mesh screen. Samples were dried in oven at 45°C and grind them and storage to environmental temperature to determine protein, lipids, fiber and ashes using Official Methods of Analysis of AOAC International [21].

2.5 Statistical analysis
A database was created using Excel 2013 software to make first analysis using descriptive statistics. All statistical analysis was made using SYSTAT 13 software (SPSS, Chicago, IL, USA). Water quality parameters and nutritional content values were analyzed by one-way ANOVA analysis. It was considered significant differences at P<0.05 level. When significant differences were observed, a Tukey test was made to identify differences between experimental groups.

3. Results
3.1 Water quality parameters
The water quality parameters monitored during experimental period did not showed significant difference for pH, nitrite, nitrate, but there was significant difference in dissolved oxygen between control and treatments, control treatment show the highest concentration (9.16 ± 3.48), all parameters showed tolerable levels for the cultivation of species in Biofloc except for temperature which is considered lower than recommendable, around 20°C (Table 1).

Table 1: Water quality parameters registered in Biofloc cultures produced with different carbon sources.

Variable	Control	Rice bran	Coffee waste	Molasses
Temperature (°C)	20.32 ± 1.00	20.34 ± 1.09	19.55 ± 3.33	20.54 ± 1.06
OD (%)	9.16 ± 3.48^a	7.55 ± 1.27^b	7.78 ± 1.96^b	7.89 ± 1.40^b
pH	7.95 ± 0.25	7.84 ± 0.25	7.90 ± 0.26	7.91 ± 0.21
NH_4^- N (mg/L)	1.82 ± 3.25	1.52 ± 2.26	1.02 ± 1.05	1.23 ± 2.54
NO_2^- N (mg/L)	4.06 ± 4.72	2.46 ± 3.26	1.86 ± 2.89	2.06 ± 3.13
NO_3^- N (mg/L)	19.70 ± 15.9	15.66 ± 13.12	16.24 ± 14.56	13.38 ± 10.83

Note: Each value represents the mean and standard deviation. Mean values in same row with different upper index show significant differences (P < 0.05).

3.2 Planktonic characterization
From the third week, microorganism's colonization began. Principal groups observed were: microalgae, protozoa, ciliates and rotifers.

3.2.1 Biofloc with rice bran
In Table 2 there is information about abundance (org mL^{-1}) of observed plankton in treatment with RB. Phytoplanktonic gender were only observed until week 6, being *Eudorina* genus the one that presented more abundance with a mean of 250 org mL^{-1} during weeks 4 and 5, as to ameboid and flagellates only *Amisonema* and *Peranema* remained until week 12 of experimentation. Regarding to ciliates, it was observed that genus *Podophyra*, *Litonotus*, *Paramecium*, *Tokophyra* and *Vortichella* were maintained until the end of experiment, on other hand, it was identified three genera of rotifers: *Philodina*, *Lecane* and *Lepadella*, being the last one, which predominated during all experiment, reaching a maximum population of 180 org mL^{-1} during week 4. Genus *Lecane* was only observed during a period of seven weeks.

Table 2: Planktonic abundance in Biofloc culture with rice bran as carbon source since third experimental week.

Plankton	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Phytoplankton										
Cianobacteria	173	84	46	17	-	-	-	-	-	-
Eudorina	84	248	253	80	-	-	-	-	-	-
Coleastrum	73	128	89	28	-	-	-	-	-	-
Ameboids and Flagelata										
Amoeba	146	33	148	-	-	-	-	-	-	-
Heliozoo	0	12	61	-	-	-	-	-	-	-
Actinophrys	19	65	51	-	-	-	-	-	-	-
Amisonema	25	105	61	179	72	166	40	34	24	20
Peranema	81	220	128	114	74	99	73	86	56	35
Ciliata										
Euplotes	-	36	99	121	181	190	48	23	17	9
Ephistilys	-	-	69	31	54	32	6	-	-	-
Podophyra	59	201	101	113	111	81	74	82	52	33
Acineta	-	-	91	64	89	76	44	58	55	35
Litonotus	60	66	24	75	74	57	56	59	45	3
Paramecium	30	167	86	88	73	93	37	11	16	15
Tokophyra	10	65	67	80	30	48	57	28	3	3
Coleps	-	24	33	52	41	49	34	32	24	-
Aspidisca	-	-	-	24	18	28	26	13	5	-
Vortichella	81	228	105	85	173	74	25	17	12	33
Rotifers										
Philodina	61	63	36	54	62	51	23	33	43	61
Lepadella	98	180	107	91	78	79	48	40	24	21
Lecane	-	53	51	42	30	37	23	28	-	-

3.2.2 Biofloc with coffee waste

In this treatment the phytoplanktonic genus *Eudorina* and *Colesastrum* were observed during three weeks with a maximum density of 107 and 104 org mL^{-1} respectively, while cyanobacteria were observed during a period of four weeks, regarding to minor protozoa (amoebas and flagellates) their apparition and permanence was intermittent, being flagellate *Paranema* which was observed most often reaching its maximum population for 4 weeks (Table 3). With respect to ciliates, seven genders were observed, but only *Litonotus*, *Paramecium* and *Vortichela* were maintained from the beginning of colonization until end of experiment. Among rotifers, *Lepadella* genus was the most dominant with initial counting above 90 org mL^{-1} but increasing up to 114 org mL^{-1} during week 5. Their mean value was 74 org mL^{-1} throughout the experiment. *Philodina* genus appeared from 5 week (50 org mL^{-1}) remaining until 11 week (43 org mL^{-1}).

Table 1: Planktonic abundance of Biofloc culture with coffee waste as carbon source since third experimental week.

Plankton	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Phytoplankton										
Cianobacteria	91	97	62	26	-	-	-	-	-	-
Eudorina	98	107	85	-	-	-	-	-	-	-
Coleastrum	81	104	63	-	-	-	-	-	-	-
Ameboids and Flagelata										
Amoeba	53	38	28	-	-	-	-	-	-	-
Amisonema	-	35	-	94	54	64	47	-	-	9
Peranema	82	175	60	48	35	42	21	0	0	49
Ciliata										
Podophyra	32	172	48	39	43	42	26	41	41	-
Acineta	-	-	51	40	82	102	15	18	18	16
Litonotus	83	33	19	24	21	22	21	22	22	10
Tokophyra	-	-	-	59	46	55	32	20	16	2
Paramecium	102	73	106	56	52	34	0	12	16	7
Coleps	-	120	82	87	87	84	59	49	24	10
Vostichella	79	199	56	52	59	49	26	19	12	25
Rotifers										
Philodina			50	40	37	35	32	26	43	0
Lepadella	94	66	114	74	78	99	88	78	24	26
Mniobia	77	67	33	79	82	59	27	62	0	15

3.2.3 Biofloc with molasses

The summary of observed microbial communities in this treatment is shown in Table 4. As in other two treatments, phytoplanktonic communities was represented by *Eudorina*, *Coleastrum* and cyanobacteria; and it was limited to first four weeks of colonization. The flagellate with more abundance was *Amisonema* reaching its maximum in weeks 6 and 8 with 152 and 147 org mL^{-1} respectively, meanwhile *Heliozoo* and *Actinophyrus* genus were only observed in weeks 4 and 5. Eight genus of ciliates were observed, five in 3 week to 12 week, where *Paramecium* was predominant with 47 org mL^{-1} in initial counting, with maximums of 133 and 114 org mL^{-1} in weeks four and seven with consecutive decrease from last one. In this treatment it was observed two gender of rotifers, *Lepadella* since 3 weeks and *Philodina* since 4 week, both were maintained until last week of experimentation.

Table 2: Planktonic abundance of Biofloc culture with molasses since third experimental week.

Plankton	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Phytoplankton										
Cianobacteria	98	48	39	33	-	-	-	-	-	-
Eudorina	48	72	93	36	-	-	-	-	-	-
Coleastrum	62	96	83	23	-	-	-	-	-	-
Ameboids and Flagelata										
Amebas	92	33	87	-	-	-	-	-	-	-
Heliozoo	-	27	91	-	-	-	-	-	-	-
Actinophrys	-	11	57	-	-	-	-	-	-	
Amisonema	17	128	48	152	100	147	67	56	54	44
Peranema	-	98	63	116	72	102	70	97	76	22
Ciliata										
Podophyra	-	122	-	113	112	110	50	28	23	22
Acineta	9	37	145	53	86	90	17	21	25	7
Litonotus	44	78	45	51	62	34	19	20	18	13
Tokophyra	-	-	-	82	81	77	56	16	14	17
Paramecium	47	133	109	96	114	66	46	34	28	29
Coleps	11	87	38	61	63	49	9	-	-	-
Aspidisca	-	-	-	46	29	35	40	26	12	0
Vostichella	129	113	38	69	113	69	39	29	24	27
Rotifers										
Philodina	-	132	19	61	58	58	59	40	25	30
Lepadella	80	153	70	58	46	44	54	43	10	28

3.3 Bioflocs proximal analysis

Proximal analysis results to different type of bioflocs are shown in Table 5. It was not observed significant differences regarding to raw lipids, but in percentage of raw fiber, CW treatment was significantly superior to other treatments and organic matter content, in this treatment was also the highest (93.3 ± 1.6) having significant difference regarding to MOL treatment (16.46 ± 2.72) and was significantly different form CW treatment. The treatments RB and MOL presented high protein levels (47.99 and 42.01% respectively). With respect to mineral content in bioflocs, there were not significant differences as to Ca, Mg and Fe, while for Na the CW treatment registered highest values (33.66 ± 11.02) showing significant difference regarding to MOL treatment and for K, content was significantly higher in MOL treatment.

Table 5: Biofloc proximal analysis produced from different carbon sources.

Composition	Commercial food	Rice bran	Coffee waste	Molasses
Fat (%)	5	2.16 ± 0.60	1.96 ± 0.31	2.50 ± 0.45
Protein (%)	35	47.99 ± 2.78 ^{a}	30.28 ± 5.33 ^{b}	42.01 ± 4.93 ^{a}
Crude fiber (%)	6	3.93 ± 0.94^{b}	29.10 ± 4.90^{a}	5.11 ± 0.97^{b}
Organic matter	n/d	88.2 ± 3.2^{a}	93.3 ± 1.6^{a}	83.5 ± 2.7^{b}
Ashes (%)	5	11.82 ± 3.23^{a}	6.71 ± 1.60^{b}	16.46 ± 2.72^{a}
Na (mEq/l)	-	19 ± 4.58^{a}	33.66 ± 11.02^{b}	7.62 ± 2.89^{a}
Ca (mEq/l)	-	6.23 ± 0.36	4.17 ± 0.38	5.73 ± 2.20
Fe (mEq/l)	-	0.16 ± 0.02	0.14 ± 0.06	0.10 ± 0.03
K (mEq/l)	-	21.00 ± 6.56^{a}	7.33 ± 1.53^{b}	38.67 ± 16.17^{a}
Mg (mEq/l)	-	17.24	7.11	18.11

Note: Each value represents mean and standard deviation. Mean values in same row with different upper index shown significant differences ($P < 0.05$).

4. Discussion

During the development of this study in different treatments (Rice bran, coffee wastes and molasses), water quality parameters were maintained in an adequate interval for tilapia culture, regarding these, diverse investigators match that Biofloc technology application offer the possibility to maintain culture water quality in optimal levels for the specie, without need to make water change [2, 4, 5, 22, 23]. The formation and development of flocs in high C/N relations, are directly related to assimilation of nitrogenous compounds [24]. On other hand, it is mentioned that ammoniacal form is tightly correlated to pH, highlighting that in high pH non-ionized nitrogen form (NH_3) is dominant, the registered pH values during this experiment (7.90-7.95) benefited the dominance of ammonium in its non-toxic form [25]. Other authors mention [26] that water quality in heterotrophic production systems based in microorganisms, are more stable that those systems based on phytoplankton. Also, other authors [27, 28] match that microorganisms associated to Biofloc play a significant role in maintenance of water quality because they participate in metabolizing wastes form feces and non-consumed food, decreasing nitrogenous compounds, mainly the toxic forms (NH_3 and NO_2^-). In this experiment this tendency was shown, because since colonization of microorganism it was observed a decrease of nitrogenous compounds, without the need of making water changes, maintaining optimal levels in treatment with rice bran, coffee wastes and molasses as carbon source to promote the production of flocs. Crab *et al* [7], mention that the carbon source used for the promotion of the flocs stimulates specific bacteria, protozoa and algae and therefore influences the microbial composition of the flocs as well as their nutritional properties. In this study the principal groups of observed microorganism in different treatments were microalgae, protozoa, ciliates and rotifers, represented by 16 genus in CW treatment, 18 genus in MOL treatment and 22 genus in RB treatment; regarding to phytoplanktonic microorganisms, in the three treatments it was only observed cyanobacteria as well as *Coleastrum* and *Eudorina* genus which obtained the highest abundance in RB treatment (253 org mL^{-1}) and were observed from third to sixth week of experimentation. In another study [29], using molasses as carbon source, they mentioned the appearance and permanence of microalgae since beginning of experimentation until the end, with densities above 600 org mL^{-1}; also, other authors [30] using a combination of rice bran and molasses as carbon source, indicated chlorophytes presences (10 week), diatoms (9 week) and cyanobacteria (4 week) with counts up to 900 org mL^{-1} for diatoms. These differences can be attributed to the variations of the light due to the constant aeration in the system, becoming a limiting factor for the development of cyanobacteria and favoring the growth of chlorophytes and diatoms [31]. On the other hand, dominant phytoplanktonic organisms at the beginning of colonization in Biofloc systems tend to decrease and disappear as it matures. Another important aspect of the phytoplanktonic organisms is its nutritional values, it is known that this value is related to environment where it develops and with inorganic compounds that they use for its growth [11], some species frequently observed in Biofloc can provide good protein, lipids and carbohydrates content [32]. Regarding to amoeboid and flagellates, in this experiment it was observed in addition to naked amoebae, related microorganisms to *Heliozoo* and *Actinophrys* genders, as well as flagellates *Peranema* and *Amisonema*, the presence of this microorganisms is clearly associated to a good efficiency in elimination of nitrogenous compounds [33], issue that was reflected in low levels of these compounds during development of this experiment. Regarding to ciliates, in this work it was identified 10 gender in rice bran treatment, similar to reported in other study [29], where nine different genus were identified in a Biofloc with molasses as carbon source; in a study made with molasses and rice bran, it were only observed five genus [30]. The ciliates are common species in Biofloc systems [13] and indicate that salinity, in the culture time and the used carbon source can affect the concentration and diversity of ciliates in water, such microorganisms play an important role in aquatic microorganism nutrition, mainly in the firsts phases of culture due to its size and protein content [34], likewise they are in charge of controlling bacterial communities density throughout depredation and be part of food web for superior zooplankton [35].

With respect to rotifers, it were observed four genus of *Mniobia*, *Lecane*, *Philodina* and *Lepadella*, with maximum concentrations of 82, 53, 132 and 180 org mL^{-1} respectively, these last two observed in the three treatments, meanwhile, *Mniobia* was only observed in CW treatment and *Lecane* in RB treatment, the quantity of observed genus is similar to the reported in other work [30], which in Biofloc with molasses and rice bran reported three genus with a concentration up to 287 org mL^{-1} and indicate that is possible that rice bran propitiate more favorable conditions for planktonic community abundance, increasing the number of species and individuals. Rotifers are frequently associated to Biofloc fulfilling some fundamental functions such as flocs fragmentation, consuming bacteria and produce mucilage [34] that helps to new flocs formation, besides these organisms can contain between 54 and 60% of protein [17].

Proximal analysis made to different Bioflocs types show variations in nutritional content of these depending in used carbon source for its promotion, highest percentage of protein was obtained with RB treatment (47%), nevertheless, it was not found significant difference regarding to obtained with MOL (42%), although in RC treatment, protein percentage was lowest (30%), such value can be considered as acceptable, if it is take into account that most species in aquaculture require a protein level in its diet between 20 and 50% [36], the lowest obtained value in this experiment is comparable with those reported by Wang *et al.* [37], which by using a mix of molasses, rice bran and wheat bran as carbon source reported protein values of 32%, and other authors [38] reported a percentage of 58% of protein using glycerol as carbon source plus a bacillus. Regarding to lipid content in flocs, obtained values in different treatments were between 1.96 and 2.50%, such values were higher to reported in other study [27] which indicated a percentage of 1.6% in generated flocs from dextrose, while it is below to Biofloc with starch, reaching an 8.5% [39]. It has been reported that lipids are the most important source of metabolic energy for its growth [40], many of this nutrients can be attributed to present microalgae in this type of systems, which have the capacity to produce big amounts of lipids in adequate conditions [41], the benefits of this nutritional contributions are reflected in higher growth and survival rates, improvement in food conversion factors and in general wealth being of cultured specie. In relation to carbon source used for Biofloc promotion, it has been used diverse options as sucrose [42], wheat bran [43] or glycerol and acetate [38]. Carbon sources such as molasses or dextrose are dissolve more easily in culture mediums than rice bran, liberating more quickly the carbon for microbial protein generation and mention that molasses has been widely used as promotor of bacterial growth in systems with cero water change [44], nevertheless, diverse investigators indicate that Biofloc culture system is viable with the use of sources rich in carbon of low cost (like rice bran or coffee wastes used in this experiment), also they recommend the use of carbon sources of low cost because it is an alternative that allows economic sustainability, provides an additional source of proteins and improves nutritional efficiency of culture system [38, 45]. The nutritional quality contributed by microorganisms associated to Biofloc is comparable and/or superior regarding to commercial food in terms of proteins and fats, also contribute to an adequate content of carbohydrates and ashes for its use as food in aquaculture, the bioflocs are also sources of vitamins and minerals, specially phosphorus, calcium and magnesium.

5. Conclusions

The results in this investigation bring significant advances in composition of different planktonic groups associated to Biofloc knowledge, and nutritional contribution they provide to cultivated species, highlighting that no matter the used carbon source, Biofloc contribution as natural food source *in situ*, is comparable to use of conventional diets, but without economic and ecological impact of traditional systems, mostly because water quality is improves and use of this scarce resource in world is limited.

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