



Proximal and sensory analysis of red tilapia (*Oreochromis* sp.) fed with fish tanks sediments from a Biofloc culture

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Abstract

Feeding accounts for 50%-60% of production costs in fish farming, and alternative raw materials are used to lower production costs. These raw materials can alter nutritional values and sensory aspects of the final product. The present study describes the results for the proximal composition, sensory, and microbiological analyses of red tilapia (*Oreochromis* sp.) fed with a biofloc meal (fish sediment residue). The results showed that the red tilapia biofloc meal contained protein levels over 30%, with 3% nitrogen and 6% calcium. Fish fed biofloc meal showed productive performances within the ranges established for the species. Sensory analysis revealed that the cooked product's appearance was very characteristic, presenting a slight metallic flavor and moderate juiciness. The bromatological composition analysis indicated that each 100g of tilapia contained 16 g of protein. Finally, the microbiological study guarantees compliance with the local standards and confirms that the product does not contain significant levels of microorganisms that could cause foodborne diseases when consumed by humans. In conclusion, the use of biofloc meal at a small red tilapia farm proved to be a sustainable alternative for feeding *Oreochromis* sp.

Keywords: alternative protein; floc meal; protein feed; biofloc technology; sustainable.

Practical Application: Microbial flocs produced in a red tilapia biofloc system could offer the small-scale tilapia industry a novel alternative feed, rich in protein and minerals, without compromising the zootechnical performance or the microbiological and sensory quality of the fish. The study communicates the use of an alternative tilapia diet including floc meals at a commercial scale, showing its high practical application.

1 Introduction

Feeding accounts for 50%-60% of fish farming production costs, which is approximately COP \$2,813 (USD \$ 1) for each kilogram of fish (Brú-Cordero et al. 2017). In order to develop sustainable aquaculture systems, alternative sources of high quality protein, the main limiting factor, will have to be identified to replace high-cost ingredients (Mbahinzireki et al., 2001; Bauer et al., 2012). Studies indicate that just about 20-35% of the nitrogen from fish diets is actually retained, with the remainder being lost as ammonia and organic nitrogen in feces and feed residue (Najdegerami et al, 2016). Recycling of nutrients occurs within fish tanks, and they can be used as an agricultural fertilizer or for feeding aquatic organisms at reduced scales (Hargreaves, 2013). Therefore, it is important to determine if alternative ingredients such as derived from biologically treating fish waste could be a suitable replacement ingredient for aquaculture diets, offering a sustainable option to fishmeal or even soy meal (Kuhn et al., 2010).

Biofloc technology (BFT) is a limited or zero water exchange productive system, which ensures intensification and sustainable feeding management (Crab et al., 2012). Bioflocs are heterogeneous aggregates of suspended organic particles and associated microorganism, including bacteria, fungi, flagellates, protozoans, ciliates, algae, among others (Luo et al., 2020). Bioflocs are a rich protein source (Xu & Pan, 2012), and

could be used as alternative ingredients to replace traditional protein sources used in small aquaculture production facilities (Hargreaves, 2013). Proximal analysis of biofloc meals from Nile tilapias, *Oreochromis niloticus*, showed that protein content can range from 17.92% to 24.5%, and that this depends on the feed used during the production process (Prabu et al., 2017; Mabroke et al., 2019), similar findings have been obtained for shrimps (Xu & Pan, 2012).

Bioflocs can supplement a portion of food protein for low protein feeds and also contribute to maintaining physiological functions in Pacific white shrimp, *Litopenaeus vannamei* (Ju et al., 2008; Bauer et al., 2012; Kuhn et al., 2009, 2010; Xu & Pan., 2014) and tiger shrimp, *Penaeus monodon* (Anand et al., 2014). In addition, appropriate dietary biofloc level in the diet of sea cucumbers, *Apostichopus japonicas*, improved the growth performance by accelerating protein turnover and optimizing energy allocation, proving to be a sustainable protein (Chen et al., 2018). Nevertheless, no previous studies have investigated the use of biofloc meals as alternative feeding for red tilapias cultured in BFT aquaculture systems.

Thus, the objective of this work was to describe the performance an alternative diet including a biofloc meal for feeding of red tilapia farmed in a BFT system: including proximal composition

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of the sediments and tilapia fillets and microbiological and sensory analysis.

2 Materials and methods

The study was prepared with data obtained from a commercial BFT fish farm: “El Vergel” Fish Association, localized in the municipality of Arauquita, Colombia. The facility used biofloc meal to feed red tilapias and performed all the analysis described in the present study as part of their own productive cycle and quality controls of their final product.

2.1 Experimental design

Juveniles of red tilapia (*Oreochromis* sp.), average weight 24 ± 0.1 g, were stocked in 19 polyethylene tanks (153 m^3), diameter 14 m ($n = 5000$), within a BFT system, and fed with constant artificial diet for 90 days. Details about the productive system are described in section 2.2. The artificial diet was prepared using local ingredients including a biofloc meal obtained from six months fish tanks residues from a prior batch at the commercial facility (Table 1). At the end of the feeding trial, fish were measured, and random samples of fish fillets were analyzed for its bromatological content, sensory analysis and microbiological quality.

2.2 Biofloc production

Biofloc production was carried out in a red tilapia commercial production system that implemented biofloc technology and was managed as per the methodology recommended by Avnimelech (2009). The carbon to nitrogen ratio was maintained at 20:1. Molasses with 480 g of carbon per kg was used to maintain this ratio to generate a heterotrophic environment indicated for the growth of microorganisms that convert ammonium to nitrite and subsequently to nitrate. When the biofloc became mature, indicated by the optimal amount of decantable solids (between 25 and 50 mL L^{-1} of water), the carbon to nitrogen ratio was modified to 6:1 and the amount of molasses was calculated depending on the amount of ammonia present in the production system. Agricultural or carbonated lime at a ratio of 10% of the amount of feed supplied per day was added to maintain an alkalinity above

120 ppm. High alkalinity values act as a buffer, maintaining pH at 7; this prevented intoxication of the fish by the non-ionized ammonium present in alkaline pH, which increases along with salinity and temperature (Ebeling et al., 2006). All tanks were aerated and mixed continuously using air pumps to meet the oxygen demand.

The decantable solids were removed daily for 6 months using decanters with a capacity of 1% of the total capacity of the production tank. To achieve this, external tanks were connected through pipes into which water containing high levels of solids slowly entered via an airlift system and flowed toward the surface exit. The solids remained at the bottom because of the effect of gravity and then were collected through a stopcock and subsequently dried at room temperature. The collected floc was processed as indicated by Chen et al. (2018). Biofloc meal was stored at $-20 \text{ }^\circ\text{C}$ until experimental diets were made.

Finally, biofloc meal samples were sent to a specialized food laboratory to assess moisture, ash content, crude protein, crude lipid, crude fiber, total carbohydrates, and caloric value. Water content was quantified by means of the AOAC 925.10 gravimetric method, by drying a sample in a hot air oven at $103 \text{ }^\circ\text{C}$ for 1h, and obtaining moisture content by weighting and comparing the sample weight prior and after drying. Lipid content was determined according to the acid hydrolysis method, AOAC 920.85 – Soxhlet extraction. Total protein content was determined using GOMEPL.01 - Kjeldahl method procedure. Ash analysis used carbonization in a muffle furnace at $550 \text{ }^\circ\text{C}$ (AOAC 923.03). Fiber was determined by enzymatic–gravimetric method (AOAC 978.10). Energy was calculated as 16.7, 16.7 and 37.7 kJ g^{-1} of protein, carbohydrate and lipids, respectively (Association of Official Analytical Chemists, 2012). Nitrogen was measured using the Kjeldahl titrimetric digestion, while several macro and micronutrient values were assessed by means of spectrophotometry and atomic absorption.

2.3 Productive performance

Tilapias were fed the alternative diet five times a day for 90 days (Table 1), at a feeding rate of 3% of their average weight. At the end of the feeding trial, zootechnical performance parameters were evaluated as indicated in equation 1.

$$\begin{aligned} \text{Weight gain (WG)} &= (\text{Final weight} - \text{Initial weight}) \text{ (g)}; \\ \text{Daily weight gain (GWG)} &= (\text{Final weight} - \text{Initial weight}) / \text{days (g/day)}; \\ \text{Biomass (B)} &= \text{final average weight} \times \text{number of animals (kg)}; \\ \text{Feed conversion rate (FCR)} &= \text{total feed consumed} / \text{total weight produced (kg feed/kg gain)}; \\ \text{survival (S)} &= (\text{final number of fish} / \text{initial number of fish}) \times 100 (\%) \end{aligned} \quad (1)$$

2.4 Bromatological content, sensory analysis and nutritional composition

After 90 days of production, five fishes from each tank were sent to an external laboratory to determine their nutritional profile. Samples were dried in an oven at $105 \text{ }^\circ\text{C}$ until constant weight, then ground and analyzed for crude protein, crude lipid, and ash contents using the standard methods as used for the biofloc meal (Section 2.2).

Table 1. Proximal composition of the diet.

Raw material	%
Biofloc meal	20.2
Blood meal	19.2
Rice meal	13.0
Cornmeal	6.5
Viscera meal	10.4
Tallow	3.3
Wheat bran	19.5
Soy cake	7.2
Mineral premix and Vit	0.7
TOTAL	100

Sensory evaluation was carried out with a trained panel of three persons to evaluate appearance color, odor, flavor, and juiciness. The global score was obtained by consensus in two replications (Amerine et al., 1965). A 0-10 scale was used. All analyses on cooked tilapia fillets were performed at optimal cooking time (70 °C), monitoring through a spit-type thermometer. The samples were divided into 3 × 3 cm sizes and packed in aluminum foil.

Additionally, microbiological evaluations were performed, including counts for *Escherichia coli*, coagulase-positive *Staphylococcus aureus*, *Salmonella* spp., and *Vibrio cholerae*. Bacteriological examinations were conducted immediately after sampling and followed the criteria described by Gatti et al. (2014).

2.5 Water quality

The methodology indicated by Delgado et al. (2018) was used for the measurement and recording of water parameters. Temperature, dissolved oxygen concentration, and saturation parameters were measured daily using a YSI EcoSense® DO200A probe. pH, total dissolved solids (TDS), and electrical conductivity (EC) were measured using the Hanna® model 991300 equipment. Chemical parameters, ammonium, nitrite, nitrate, alkalinity, and orthophosphates were evaluated once a week using colorimetric established methodologies. Finally, the volume of total decantable solids (DS) was measured using an Imhoff cone (American Public Health Association, 2005).

3 Results and discussion

Numerous studies have reported complete or partial replacement of high cost ingredients such as fishmeal with plant-based ingredients in diets for omnivorous fish and prawns. On the other hand, few studies have addressed biofloc meals as ingredients in aquaculture diets, with most studies focused on prawns. Kuhn et al. (2009, 2010) reported high protein content for *L. vannamei* biofloc meals, showing that the alternative diets improved shrimp growth by an average 11-49% compare to a control diet. A recent study demonstrated that dietary supplementation of biofloc meal at 4-8% level had beneficial effects on growth performance and digestive enzyme activities in *P. monodon* (Anand et al., 2014). The present study relates the use of a biofloc meal as an alternative ingredient for red tilapias farmed in a BFT system.

The results of macronutrient and micronutrient analyses (Table 2) showed that the protein levels of the biofloc meal were above 30% and that the macronutrient with the highest presence in this product was nitrogen (3.03%). Protein content was higher than reported by Bauer et al. (2012) for microbial floc meal obtained from shrimp BFT tanks (23.39%) and by Prabu et al. (2017) for GIFT tilapias (17.92%). In addition, protein levels were similar to those found by Luo et al. (2017) for Nile tilapia bioflocs prepared with different carbon sources. Differences in the protein content of flocs can be related to the nitrogen levels and nitrifying reactions by microorganisms because this nitrogen-reducing action can be evaluated by measuring protein levels in sediments (Luo et al., 2019).

Table 2. Bromatological and nutrient analysis of the biofloc meal (red tilapia sediment residues).

Bromatological proximal analysis		
Parameters	Unit	Value
Humidity		10.78
Ash		30.59
Fat		0.40
Protein	%	30.26
Fiber		4.44
Total carbohydrates		27.98
Caloric Value		236.51
Micro and Macronutrient Values		
Nitrogen		3.03
Phosphorous		0.97
Potassium		0.30
Calcium		6.96
Magnesium		0.24
Manganese	%	<0.01
Iron		0.64
Copper		<0.01
Zinc		0.09
Boron		<0.01
Sulfur		0.92

In fish farming, heterotrophic bacteria directly and rapidly convert accumulated ammonia into bacterial protein, which is reflected in the levels of this product in the sediments (Ebeling et al., 2006). Moreover, microalgae, protozoa, rotifer, oligochaeta detritus and dead organic particles are constantly observed in the sedimentable flocs and can serve as protein sources (Azim & Little, 2008; Wei et al., 2020).

The high percentage of ash found in the biofloc meal of the present experiment (30.59%) can be explained by the high salt content in the BFT system and has been observed before in floc meals collected from *L. vannamei* BFT tanks (Ju et al., 2008; Bauer et al., 2012) and Nile tilapia BFT tanks (51.28%) (Prabu et al., 2017). Additionally, lipid levels found in this study were low, indicating that the tilapia growth was mostly associated to other components from the biofloc meal.

It is estimated that 88% of the nitrogen accumulated in a fish farming system is related to feed. One way to remove this nutrient is through sediments that represent approximately 2.6% of nitrogen (Gross et al., 2000). However, the raw protein in shrimp feed does not significantly affect the accumulation of nitrogen in the system (Prangnell et al. 2016), but, in tilapias biofloc systems, nitrogen increases with the levels of protein present in the feed (Azim & Little, 2008). In the present study, 3.03% of nitrogen was found in the biofloc meal (Table 2). This is similar to that found in sludge from *O. niloticus* and rainbow trout, *Oncorhynchus mykiss*,

production systems where the reported nitrogen was 2.74% (Ingle de la Mora et al., 2003).

Calcium was the nutrient with the highest presence in the residual sediment collected over 6 months of red tilapia production in BFT (6.96%). This result was lower than reported for *O. niloticus* diets with inclusion of 25 or 50% floc meal (Mabroke et al., 2019). The variations in the results may be related to the fact that different levels of calcium carbonates are supplied in the biofloc system to maintain alkalinity levels, which is reflected in the levels of this nutrient in the sediments (Furtado et al., 2014). Nutrients are recycled and can accumulate owing to little or no water exchange in the biofloc system (Martins et al., 2017).

The zootechnical performance of tilapias fed with diets containing fish sediment meal (Table 3) were similar to those reported by Prabu et al. (2017), mainly in the FCR when feeds for Nile tilapia were enriched with 20% biofloc and lysine. The best FCR results were observed using 20% biofloc and 1.2% lysine (1.06), and the lowest using 20% biofloc and 1.8% lysine. In addition, FCR values were superior than related by Mabroke et al. (2019) for Nile tilapias fed with diets including of 25 or 50% floc meal for 56 days. Nevertheless, it was found that the amounts of sediment are not constant enough for large-scale production. In sea cucumbers, *A. japonicus* fed with increasing levels of biofloc meals, a remarkable downward trends on zootechnical parameters was observed as dietary biofloc level increased from 30% to 60% (Chen et al., 2018). Therefore, future studies addressing different proportions of biofloc meals for red tilapias should be designed.

Nutritional composition analyses (Table 4) indicate that tilapias fed with fish sediments had moisture and protein levels of 72.28% and 16.56%, respectively. Results are in agreement with prior values obtained for red hybrid tilapia fillets (Garduño-Lugo et al., 2007). Additionally, results are similar to those reported by Carneiro et al. (2017) in Nile tilapias fed diets containing 30% digestible protein based on soybean and corn meal, resulting in 16.2% of body protein. However, crude lipid in our study were higher (7.8%) than those found by Carneiro et al. (2017) in Nile tilapias (4.1%). This fact can be explained by the use of tallow, and not soybean oil, in the formulation of the present study.

The microbial flora of fish depends specifically on the microorganisms present in the water where they are grown, the feed used, or the slaughter process used (Boari et al., 2008). *Staphylococcus aureus* and *Escherichia coli* are not natural microbiota of fish and can be associated with fish fillets as a consequence of poor water quality or contamination during the food production chain (Roberts et al., 2005; Gatti et al., 2014). The analysis of microbiological requirements (Table 5) determined that tilapias fed with fish sediments comply with the local regulations for *S. aureus* and *E. coli* counts on fish fillets (Suarez, 2017). *Salmonella* spp. and *Vibrio* spp. could cause foodborne diseases, and are present in regions with high water temperature or polluted waters (Huss, 1994). Satisfactory, in the present study none of such bacteria was found in the red tilapia fillets. The results of this study confirm that the final product can be consumed and that the drying and grinding process performed with the

Table 3. Productive performance parameters of red tilapia fed with a diet containing biofloc meal.

Parameter	Result
WG g	166 ± 6
DWG g/d	1.84 ± 0.06
Biomass kg	186.2 ± 9.8
FCR	1.23 ± 0.03
S %	98

WG: Weight gain, DWG: Daily weight gain, FCR: Feed conversion rate, S: Survival.

Table 4. Bromatological analysis of tilapias fed with a diet containing biofloc meal.

Parameters in g/100 g (%)	Result
Moisture	72.28
Ash	2.78
Crude lipid	7.80
Crude protein	16.56
Total carbohydrates	0.58
Calories	138.74
Raw fiber	ND

ND: Non-detected.

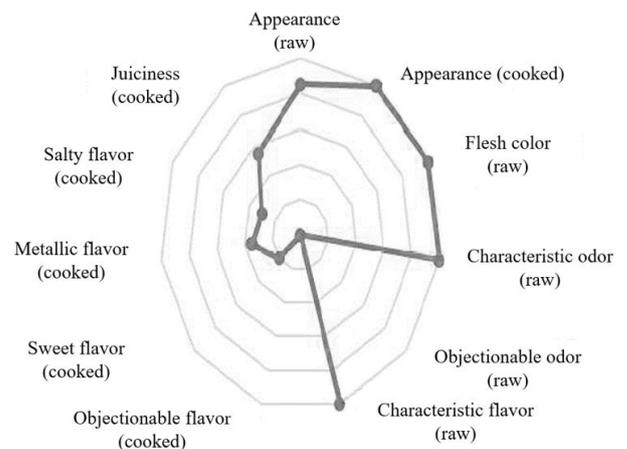


Figure 1. Intensities of sensory descriptors for tilapias fed with a diet containing biofloc meal.

fish sediments reduced the risk of contamination with human pathogenic microorganisms.

The sensory technical data sheet (Table 6, Figure 1) showed that the characteristic flavor as well as the appearance was very strong when the product was cooked. These results can be affected by storage and preparation. However, it was evident that the results are similar to those reported for red hybrid tilapias fed with alternatives ingredients to fish oil, where it was concluded that this factor did not affect the sensory attributes of the product (Ng & Bahurmiz, 2009).

Table 5. Microbiological analysis of red tilapias fed with a diet containing biofloc meal.

Analysis	Criterion or specifications	Results	Compliance
<i>E. coli</i>	10-400 UFC/g	40	Complies
Coagulase-positive <i>Staphylococcus aureus</i>	100-1000 UFC/g	<100	Complies
<i>Salmonella</i> spp.	Absence/25 g	Absence	Complies
<i>Vibrio cholerae</i>	Absence/25g	Absence	Complies

Table 6. Intensity rating of sensory descriptors for tilapias fed with a diet containing biofloc meal.

Descriptors	Product		Whole gutted and scaleless tilapia	Intensity
	Cooked	Raw		
Appearance		x	8.5	Strong
	x		10	Very strong
Flesh color		x	10	Very strong
Characteristic odor		x	10	Very strong
Objectionable odor		x	0.0	Absence
Characteristic flavor	x		10	Very strong
Objectionable flavor	x		0.0	Absence
Sweet flavor	x		2.0	Very mild
Metallic flavor	x		3.5	Mild
Salty flavor	x		3.0	Mild
Juiciness	x		5.5	Moderate

Table 7. Water quality parameters during the feeding trial.

Parameters	Reference values (Boyd & Massaut, 1999)	Present study
Dissolved oxygen (ppm)	>3	4.1 ± 0.1
Oxygen saturation (%)	50-70	60.1 ± 8.3
Temperature (°C)	24-30	27.4 ± 1.2
pH	6.5-8	7.5 ± 0.3
TDS (ppm)	<1000	990 ± 123
EC (µScm ⁻¹)	<1000	832 ± 67
Ammonia (ppm)	<1	0.2 ± 0
Nitrite (ppm)	<1	0.1 ± 0
Nitrate (ppm)	10-200	10 ± 0.4
Orthophosphates (mgL ⁻¹)	1-10	0.254 ± 0.1
Alkalinity (ppm)	>120	122 ± 16
DS (ppm)	25-50	21 ± 0.4

TDS: Total dissolved solids, EC: electrical conductivity, DS: decantable solids.

The water quality parameters (Table 7) were within the ranges indicated for the species (Boyd & Massaut, 1999). The nitrogenous compounds were not affected by the diet, which could possibly be owing to the continuous water exchanges, which did not allow the accumulation of these nutrients in the water.

4 Conclusion

The present study relates that red tilapia sediments from BFT tanks have appropriate levels of protein, and that the zootechnical performance of red tilapias fed with diets containing the biofloc meal was adequate for the species and the production stage. In addition, the raw feed did not interfere with the microbiological, bromatological and sensory quality of the final product. However, the amounts of sediment are not constant and therefore are not sufficient for commercial-scale feed production, but they can be used as a supplement in small fish facilities. Finally, further studies are needed including comparisons between common commercial diets for tilapia and different levels of inclusion of the biofloc meal.

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