Latin American Journal of Aquatic Research, 52(2): 289-297, 2024
DOI: 10.3856/vol52-issue2-fulltext-3142

Research Article

Partial substitution of fish meal by biofloc meal in diets for Nile tilapia *Oreochromis niloticus*

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ABSTRACT. The effect of a partial substitute of fish meal by biofloc meal on the growth and survival of fry Nile tilapia *Oreochromis niloticus* was evaluated over nine weeks. Two biofloc meals were obtained by drying them in two processes: A) sun exposure and B) convection oven. A randomized design of eight isonitrogenous (40%) and isoenergetic (17 kJ g^{-1}) diets was used, where 5, 10, 15, and 20% of the fish meal was substituted for the two biofloc meals. The nutritional study was conducted in a recirculating aquaculture system with Nile tilapia fry (initial weight: 0.64 ± 0.16 g). The survival range was similar in all treatments and higher than 97%. Tilapia fed with the treatments A5, A10, B5, and B10 of substitution of biofloc meal showed similar and significantly better final weight, individual weight gain, specific growth rate, and condition factor than A15, A20, B15, and B20 treatments; as well as efficiency of use of the feed: protein efficiency ratio, carcass nitrogen deposition, apparent nitrogen utilization, and apparent digestibility. Therefore, these results suggest that biofloc meal is possible in diets for tilapia, thus reducing the need for fishmeal as the main source of protein in the diet.

Keywords: *Oreochromis niloticus*; alternative protein; tilapia nutrition; microbial protein; dietary supplement; digestibility; aquafeed

INTRODUCTION

The growing world population and the increased demand for aquatic foods intensify production systems (FAO 2022). Although fisheries are an important segment of the global food system, sustained growth is not anticipated in the future (Boyd 2015); aquaculture is the only way to guarantee aquatic food supply to a continuously growing population (Stickney 2005).

To ensure aquaculture's growth, the constant and increasing use of balanced feed is required; therefore, the aquafeed industry has to grow at an average rate of 7.7% per year (Tacon et al. 2022); however, providing a large quantity and quality of aquafeed will be one of the crucial challenges of this industry (Khanjani et al. 2023).

Protein is the most important nutrient in aquafeeds (Li & Robinson 2015). Usually, it comes from fishmeal because its nutrient profile is close to the nutrient requirements of most farmed species (Khanjani et al. 2023). The percentage of captured fish that is processed into fishmeal has decreased in recent decades, causing demand and prices to increase (FAO 2022); therefore, alternative ingredients are required to totally or partially replace fishmeal, for which its nutritional value, digestibility, production process, physical presentation, availability, impact on food, and economic effect of its use in commercial-scale crops must be evaluated (Engle 2017).

Research aimed at substituting fishmeal in aquafeeds with alternative ingredients includes evalua-

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Associate Editor: Fernando Vega

tion of meals derived from fish and terrestrial animal by-products, meals and by-products from oilseeds, aquatic plants, single cell protein, and by-products from legumes and cereals (Engle 2017); however, these resources have their limitations, for example amino acid deficiencies, anti-nutritional factors, low digestibility and palatability compared to fishmeal (Khanjani et al. 2023). In this sense, the level of inclusion of alternative protein ingredients in aquaculture diets varies according to the source; for example, plant-derived protein ingredients can be included from 5 to 60%, protein ingredients of animal origin between 5 and 15%, and protein sources derived from pure strains of microorganisms (microalgae, yeasts, and bacteria) from 5 to 30% (Tacon et al. 2012, Gambo-Delgado & Márquez-Reyes 2016).

Recently, Khanjani et al. (2023) indicated that biofloc, a by-product of the biofloc technology culture system, can be considered an alternative source of protein to fishmeal or a practical additive in aquafeeds. The biofloc is a mixture of microalgae, bacteria, fungi, protozoa, metazoa, and particulate organic matter (Gallardo-Collí et al. 2019). They are the source of biofloc macronutrients, providing between 23 and 42% of proteins and between 0.5 and 12% of lipids, in addition to bioactive components: carotenoids, chlorophyll, phytosterols, bromophenols, amino sugars, free amino acids and vitamins (Ju et al. 2008a, Martínez-Córdova et al. 2017).

One species that take advantage of the "native" biofloc to cover part of its daily nutrient requirement is the Nile tilapia *Oreochromis niloticus* (Pérez-Fuentes et al. 2018). This cichlid has physiological adaptations that facilitate the consumption and digestion of biofloc components (Avnimelech 2007), which allows us to assume that Nile tilapia could take advantage of biofloc meal if it is integrated into a diet as a protein alternative to fishmeal. Given the importance of the aquaculture sector in the production of aquatic foods for human consumption and the urgent need to find protein ingredients that can totally or partially replace fishmeal in aquaculture feeds, the purpose of this research is to evaluate biofloc meal as a protein ingredient in diets for Nile tilapia.

MATERIALS AND METHODS

Experimental design

The study was conducted over nine weeks in the Genetic Improvement and Aquaculture Production Laboratory and the Investigation Laboratory in Aquaculture Biotechnology of the Technology Institute of Boca del Rio, Veracruz, Mexico. A completely randomized one-factor design was used with eight treatments, each with three replicates and a control.

Biofloc meal

The "native" biofloc was produced from semi-intensive tilapia culture with biofloc technology when the fish were fed a diet of 32% protein and 5% lipids, and during the culture, a C:N ratio of 12.5:1 was maintained using molasses as a carbon source, reaching a final biomass of 7.9 kg m^{-3} after of 144 days of culture (final weight 166.3 ± 35.5 g). The water quality characteristics of effluent were: temperature 25.5°C, dissolved oxygen 4.4 mg L^{-1}, pH 7.3, settleable solids 24.4 mL L^{-1}, and NH_4 0.30 mg L^{-1}.

The biofloc meal was obtained by treating the effluents (50 m^3) of tilapia culture in biofloc. For this, a three-stage production protocol was applied: thickening by sedimentation, where the water with biofloc was sedimented for 1 h and the supernatant was siphoned; dewatering by centrifugation, where the residual sludge was centrifuged at 710 rpm for 15 min, using a rotor and 50 µm cloth sleeve, adapted to a home washing machine (Samsung WA14H6-1, Samsung Electronics Co., Suwon, KR); and drying by sun exposure (A), where the biofloc paste was in direct exposure for periods of 8 h (09:00 a 17:00 h), and drying by convection oven (B) with forced air circulation (Memmert UFP 400, Schwabach, DE) at 60°C (Show et al. 2019). The result was two biofloc meals, A and B, that differ in drying.

Proximate composition of the biofloc

The proximal composition of the biofloc meals, A and B, was determined by: crude protein (%) with an elemental analyzer (Flash 2000 Thermo Fisher Scientific, Waltham, Massachusetts, USA) (N×6.25); total lipids (%) with the Soxhlet method; ash (%) from incineration 600°C in a muffle (Felisa, Jalisco, Mexico). All the analyses were conducted in triplicate using standard methods (AOAC 2000) (Table 1).

Experimental diets

Eight isonitrogenous (40% protein) and isoenergetic (17 kJ g^{-1} gross energy) diets were formulated through simultaneous equations in a Microsoft® Excel calculation page, in which 5, 10, 15, and 20% of the fish meal were substituted for A or B biofloc meal. The experimental diets were A5, A10, A15, A20, B5, B10, B15, and B20. The control diets were formulated with fish meal as the main protein source (Tables 1-2).

Table 1. Proximate composition of biofloc meal and fish meal as ingredients of experimental diets.

Proximate composition (%, based on dry weight)	Biofloc meal		Fish meal
	A	B	(El Pedregal Silver Cup feed manufacturers)
Crude protein	25.5 ± 0.41	24.3 ± 0.11	65.2
Total lipids	1.5 ± 0.32	2.3 ± 0.06	14.1
Ash	33.5 ± 0.41	35.2 ± 0.08	15.5

Table 2. Ingredient composition of experimental diets containing biofloc meal as a partial replacement of fish meal to feed Nile tilapia fry. [1]Vitamin and mineral premix: Kirkland, Vitae laboratories, Jalisco, Mx. Vitamins and minerals are present in the mixtures.

Ingredients (g kg^{-1})	Treatment								
	Control	A5	A10	A15	A20	B5	B10	B15	B20
Fish meal	613.3	582.6	552.0	521.3	490.6	582.7	552.0	521.3	490.7
Biofloc meal A	0.0	78.4	156.8	235.2	313.6	0.0	0.0	0.0	0.0
Biofloc meal B	0.0	0.0	0.0	0.00	0.0	85.1	164.7	247.1	329.5
Wheat meal	270.0	183.0	96.0	9.0	3.0	164.0	72.0	0.0	4.0
Corn starch	25.4	60.7	96.0	131.3	72.4	73.6	113.4	126.6	50.0
Canola oil	1.3	5.3	9.2	13.2	30.4	4.6	7.9	15.0	35.8
Mineral premix[1]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin premix[1]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin C	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Wheat bran	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Carboxymethyl cellulose	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0

Before the preparation of the experimental diets, the dry ingredients were pulverized at a <200 µm particle size with an electric grinder (Krups GX4100). The ingredients were mixed in a blender (Crypto Peerless, Rotabowl, Mod. JC32, England) for 25 min; oil, carboxymethyl cellulose, and hot water (60°C) were added until a homogenous paste was obtained and then passed through a mill (TOCT 4025-95, Jiaozuo Double Eagle Machinery Co) to obtain pellets of ≤1.5 mm diameter. The pellets were dried in a convection oven with forced air circulation at 60°C for 24 h, then sifted and stored at -4°C until use. Previous to use, the proximal composition of the diets was determined following standard methods (AOAC 2000) per triplicate (Table 3).

Tilapia culture

For the study, 2000 Nile tilapia fry were used, with an initial average weight of 0.64 ± 0.16 g and an initial average length 2.79 ± 0.22 cm, and were randomly distributed at a density of 0.5 ind L^{-1} in 27 cylindrical tanks of low-density polyethylene (100 L capacity) in a freshwater recirculation system with a solid separator, biological filter, reservoir and a 2 L min^{-1} water flow rate, installed in a greenhouse to maintain a natural photoperiod and constant water temperature. Before the beginning of the study, a batch of 200 tilapia fry was sacrificed with a clove oil overdose (75 mg L^{-1}) to determine their proximal composition, following standard methods per triplicate (AOAC 2000).

At the beginning of the culture, the tilapia were fed at a rate equivalent to 10% of the initial biomass in eight rations per day (09:00, 10:10, 11:20, 12:30, 13:40, 14:50, 16:00, and 17:10 h) and in the last two weeks the tilapia were fed three times a day (09:00, 13:00 and 17:00 h). The feed rate, the number of rations, and the time of feeding were adjusted each week after measuring the increase in weight (Ohaus Scout Pro C200, Balance Ohaus Co., NJ, USA; ± 0.01 g) and length (icthyometer of 30 ± 0.1 cm) of a batch of 90 randomly chosen tilapia per treatment. A mortality register was kept daily, and the dead fish were removed from the system and counted by treatment. At the end of the culture period, 15 tilapia per treatment were sacrificed with a clove oil overdose (75 mg L^{-1}) to determine their proximal composition (AOAC 2000).

During the culture, the following physicochemical parameters of the water were determined daily: temperature, dissolved oxygen, and pH (DO Meter Kit 850048, Sper Scientific; Large Display pH pen 850050,

Table 3. Proximate analyses of experimental diets containing biofloc meal as a partial replacement of fish meal to feed Nile tilapia fry. NFE, nitrogen-free extract = 100 – (moisture + crude protein + total lipids + ash). Gross energy calculated using the following factors: carbohydrate (as NFE) 17.15 kJ g^{-1}, protein 23.63 kJ g^{-1}, and lipids 39.53 kJ g^{-1}.

Proximate composition ($g\ kg^{-1}$)	Treatment								
	Control	A5	A10	A15	A20	B5	B10	B15	B20
Moisture	43	58	47	55	47	40	46	48	41
Crude protein	428	420	416	419	417	416	428	429	429
Total lipids	97	96	94	94	100	94	95	98	116
Ash	156	172	184	200	220	177	191	207	226
NFE	277	254	259	232	207	273	240	218	187
Gross energy (kJ g^{-1})	18.7	18.1	18.0	17.6	17.7	18.2	18.0	17.8	17.9

Sper Scientific), and weekly NH_4^+, NH_3^-, NO_2^-, NO_3^-, total alkalinity and total hardness (Orion AQ3700 Aquafast, Thermo Scientific).

The growth parameters and the efficiency of feed use were calculated using the following equations: survival (S, %) = (number of live fish / number of fish stocked) × 100; final weight (FW, g); individual weight gain (IWG, g d^{-1}) = weight gain (g) / time (d); specific growth rate (SGR, % d^{-1}) = 100(Ln FW - Ln IW initial weight) / time (d); condition factor (K) = 100 × [body mass (g) / $length^3$] length being in cm; feed intake (FI, g d^{-1}) = feed intake (g) / time (d); feed conversion ratio (FCR) = FI / IWG; protein efficiency ratio (PER) = weight gain (g) / protein intake (g); carcass nitrogen deposition (CND, mg d^{-1}) = 1000((FW × final carcass protein) - (IW × initial carcass protein)) / 100 / time (d) / 6.25; apparent nitrogen utilization (ANU, %) = 100 × (CND / nitrogen intake); apparent dry matter digestibility (ADMD, %) = 100 × [(DM feed ingested (g) - DM feces (g)) / DM feed ingested (g)]; and apparent crude protein digestibility (ACPD, %) = 100 × [(CP feed ingested (g) - CP feces (g)) / CP feed ingested (g)] (Smith & Trabett 2004).

Digestibility apparent in the diets

The digestibility of the diets was evaluated by the gravimetric method (Smith & Trabett 2004), in which, a week before the conclusion of the study, 15 fish per treatment were transferred to plastic containers with 20 L capacity (5 fish per replicate), and fed with the same diet, ration, and frequency as the original treatment. After seven days, the feeding rate of the fish was reduced from 3 to 1%, and the feed was administered as a single ration (09:00 h) for three days. At the end of the 30 min, the feed that was not consumed was recovered, dried, and weighed; subsequently, the tilapia feces was collected by siphon every 30 min (10:00 to 22:00 h) to reduce the leaching. The feces were dried in a convection oven at 60°C for 12 h and kept at -4°C until analysis (Chen et al. 2018).

Statistical analysis

Results were analyzed with one-way analyses of variance (ANOVA) after testing for variance normality and heterogeneity. Where the assumptions were not fulfilled, the data were $\log_{10}$ transformed, and the proportions were arcsine transformed. A Tukey test determined differences between treatments. The analysis was conducted with a nominal significance of 5% with the Statistics 10 program (Stat Soft, Tulsa, OK).

RESULTS

The physicochemical parameters of the water in the recirculation system were maintained within the tolerance ranges for tilapia with the following average values: temperature 29.56 ± 0.78°C, dissolved oxygen 6.58 ± 0.29 mg L^{-1}, pH 7.9 ± 0.4, NH_4^+ 0.09 ± 0.08 mg L^{-1}, NH_3^- 0.07 ± 0.06 mg L^{-1}, NO_2^- 0.03 ± 0.01 mg L^{-1}, NO_3^- 43.24 ± 27.10 mg L^{-1}, total alkalinity 119 ± 31 mg L^{-1} as $CaCO_3$, and total hardness 142 ± 22.38 mg L^{-1} as $CaCO_3$.

After nine weeks of the trial, tilapia survival in all treatments was similar and was higher than 97%. The average values of FW, IWG, SGR, and K, as well as PER, CND, ANU, ADMD, and ACPD of the tilapia in the experimental treatments, decreased as the biofloc meal substitution increased in the diets, where the best results were observed with the A5, A10, B5, B10, and control diets which were statistically similar but superior to the A15, A20, B15 and B20 diets that obtained the lower efficiency (Fig. 1, Table 4).

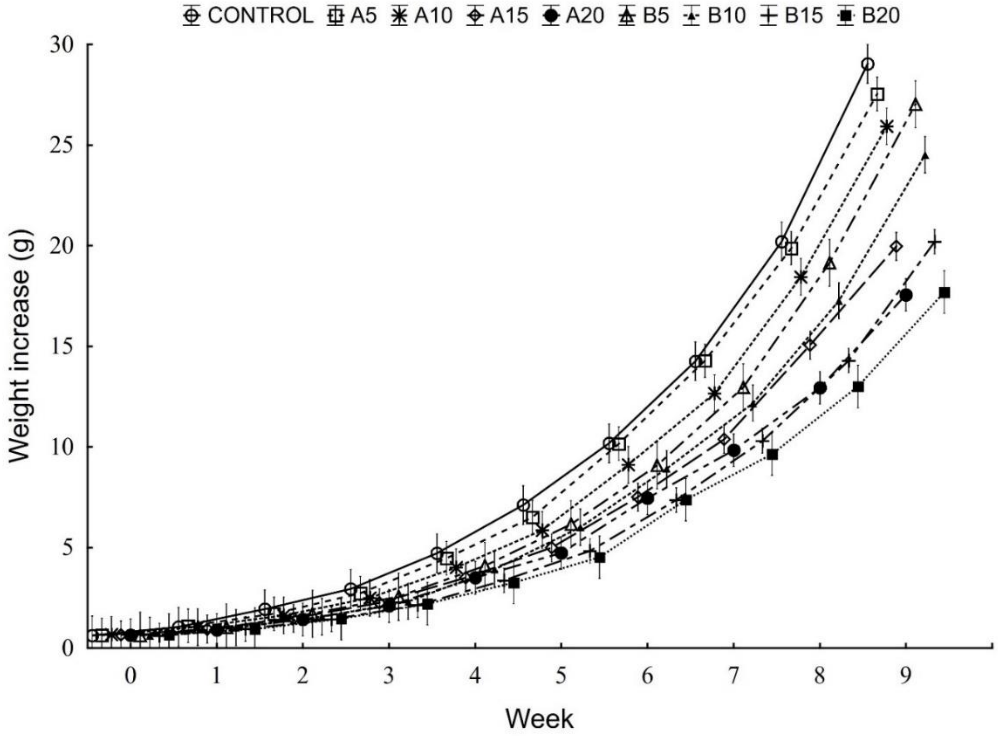

Figure 1. Growth of Nile tilapia *Oreochromis niloticus* fry-fed diets containing biofloc meal as a partial fish meal substitute. A: biofloc meal drying by sun exposure), B: biofloc meal drying in a convection oven.

The FI in the tilapia of the treatments control, A5, A10, B5, and B10, was similar and significantly superior to the tilapia of the treatments A15, A20, B15, and B20 ($P < 0.05$). The FCR of the tilapia of treatment A5 and B5 were similar and significantly lower than in treatments A15, A20, B15, and B20 ($P < 0.05$) (Table 4).

The body moisture and protein content of the tilapia subjected to treatments A15 and B20 was similar and significantly greater compared to control and treatment A5 ($P < 0.05$), similar to those observed in B5. Except for treatment B20, the lipid content of the fish was similar between the control and the evaluated treatments. The ash content in A20 and B20 was similar and significantly greater compared to the other treatments ($P < 0.05$) (Table 4).

DISCUSSION

The physicochemical parameters of the water in the recirculation system were within the acceptable range for tilapia culture (Binalshikh-Abubkr & Mohd-Hanafiah 2022); hence, the productive performance and feed efficiency observed during the study were considered effects of the experimental diets.

The survival of the tilapia in the nutritional trial was similar and high for all treatments (97 to 100%) and similar to that reported by Prabu et al. (2018), who included biofloc meal as a partial substitute for fish meal and soy meal in diets for tilapia, but greater than the survival that observed by Ekasari et al. (2018) in Nile tilapia (60%) and by Binalshikh-Abubkr & Mohd-Hanafiah (2022) with red hybrid tilapia (*Oreochromis* sp.) (80%). Recently, it has been reported that replacing conventional protein ingredients with biofloc meals achieves a survival rate similar to those fed a fishmeal-based diet (Khanjani et al. 2023).

In this study, the high survival of tilapia could be attributed to the nutrients and bioactive components (carotenoids, chlorophyll, bromophenols, phytosterols, amino sugars, vitamins) present in the biofloc meal (Ju et al. 2008a; Pérez-Fuentes et al. 2018), as with the nutritional characteristics of the fish meal, which is a source of protein of high quality, providing essential fatty acids, macrominerals, trace elements and vitamins (Khanjani el al. 2023).

The results of the present study indicate that it is possible to substitute between 5 and 10% of the fish meal with biofloc meal, which, in addition to providing a high survival rate, promoted growth increase similar

Table 4. Productive performance, feed utilization efficiency, and carcass proximate composition in Nile tilapia *Oreochromis niloticus* fry-fed diets containing biofloc meal as a partial fish meal substitute (mean ± standard deviation, SD). A: biofloc meal drying by sun exposure, B: biofloc meal drying in convection oven, S: survival, FW: final weight, IWG: individual weight gain, SGR: specific growth rate, K: condition factor, FI: feed intake, FCR: feed conversion ratio, PER: protein efficiency ratio, CND: carcass nitrogen deposition, ANU: apparent nitrogen utilization, ADMD: apparent dry matter digestibility, ACPD: apparent crude protein digestibility. Carcass proximate composition: crude protein, total lipids, ash, and NFE are based on dry weight. Initial value of carcass proximate composition: moisture = 77.77 ± 0.06%, crude protein = 67.18 ± 0.16%, total lipids = 10.69 ± 0.52%, ash = 20.47 ± 0.15%. Mean ± SD in the same row with different superscript letters indicates significant statistical differences ($P < 0.05$).

Parameter	Treatment								
	Control	A5	A10	A15	A20	B5	B10	B15	B20
S (%)	99.33 ± 0.57	100.00 ± 0.00	99.58 ± 0.72	99.66 ± 0.57	99.00 ± 1.73	98.75 ± 1.25	97.75 ± 0.25	100.00 ± 0.00	99.25 ± 0.66
FW (g)	29.03 ± 8.28^{a}	27.54 ± 6.26^{ab}	25.93 ± 6.13^{b}	19.96 ± 5.45^{c}	17.55 ± 5.24^{d}	27.02 ± 5.63^{ab}	24.52 ± 6.30^{b}	20.19 ± 5.11^{c}	17.69 ± 4.69^{d}
IWG (g d^{-1})	0.52 ± 0.45^{a}	0.49 ± 0.39^{a}	0.46 ± 0.39^{ab}	0.35 ± 0.28^{c}	0.31 ± 0.23^{c}	0.47 ± 0.41^{ab}	0.42 ± 0.35^{b}	0.36 ± 0.30^{c}	0.31 ± 0.24^{c}
SGR (% d^{-1})	5.40 ± 0.94^{a}	5.28 ± 0.89^{ab}	5.14 ± 1.04^{ab}	4.69 ± 1.10^{bc}	4.49 ± 1.24^{c}	5.27 ± 1.04^{ab}	5.10 ± 1.08^{ab}	4.79 ± 1.16^{bc}	4.47 ± 1.27^{c}
K	3.64 ± 0.77^{a}	3.58 ± 0.75^{ab}	3.54 ± 0.71^{b}	3.43 ± 0.63^{c}	3.40 ± 0.59^{c}	3.65 ± 0.84^{a}	3.56 ± 0.68^{b}	3.40 ± 0.62^{c}	3.37 ± 0.59^{c}
FI (g d^{-1})	0.50 ± 0.46^{a}	0.49 ± 0.47^{a}	0.49 ± 0.47^{a}	0.40 ± 0.35^{b}	0.38 ± 0.31^{c}	0.50 ± 0.48^{a}	0.50 ± 0.48^{a}	0.40 ± 0.35^{b}	0.38 ± 0.31^{c}
FCR	0.90 ± 0.17^{a}	0.94 ± 0.16^{ab}	1.02 ± 0.15^{bc}	1.14 ± 0.24^{cd}	1.24 ± 0.25^{d}	1.02 ± 0.24^{ab}	1.09 ± 0.20^{bc}	1.14 ± 0.22^{cd}	1.25 ± 0.27^{d}
PER	2.66 ± 0.49^{a}	2.59 ± 0.42^{a}	2.39 ± 0.37^{ab}	2.17 ± 0.41^{bc}	2.01 ± 0.44^{c}	2.45 ± 0.44^{ab}	2.20 ± 0.42^{bc}	2.10 ± 0.44^{c}	1.96 ± 0.51^{c}
CND (mg d^{-1})	46.26 ± 1.63^{a}	43.55 ± 2.29^{a}	41.95 ± 2.21^{a}	33.40 ± 1.47^{b}	27.57 ± 1.37^{c}	44.51 ± 0.29^{a}	40.01 ± 1.57^{a}	32.04 ± 1.51^{bc}	29.32 ± 2.87^{bc}
ANU (%)	33.12 ± 1.17^{a}	31.76 ± 1.67^{a}	31.12 ± 1.64^{a}	30.10 ± 1.32^{ab}	26.14 ± 1.30^{b}	32.78 ± 0.22^{a}	28.85 ± 1.13^{ab}	28.15 ± 1.33^{b}	27.04 ± 2.65^{b}
ADMD (%)	86.64 ± 1.21^{a}	84.09 ± 2.38^{a}	81.22 ± 1.96^{a}	73.56 ± 2.10^{b}	72.05 ± 2.03^{b}	83.22 ± 1.75^{a}	81.81 ± 1.18^{a}	70.86 ± 1.61^{b}	71.05 ± 1.41^{b}
ACPD (%)	91.85 ± 0.73^{a}	90.11 ± 1.47^{a}	88.87 ± 1.16^{a}	84.40 ± 1.83^{b}	83.72 ± 1.20^{b}	90.14 ± 1.03^{a}	90.20 ± 0.63^{a}	82.79 ± 0.95^{b}	83.09 ± 0.82^{b}
Carcass proximate composition (%)									
Moisture	74.33 ± 0.18^{a}	74.30 ± 0.34^{a}	74.70 ± 0.71^{ab}	76.27 ± 0.55^{b}	75.54 ± 0.44^{ab}	75.35 ± 0.39^{ab}	75.77 ± 0.39^{b}	76.23 ± 0.77^{b}	76.96 ± 0.33^{b}
Crude protein	64.18 ± 0.48^{a}	63.82 ± 1.20^{a}	65.41 ± 0.88^{ab}	68.10 ± 0.19^{b}	64.30 ± 0.37^{a}	66.42 ± 0.83^{ab}	65.93 ± 1.20^{ab}	64.52 ± 2.58^{a}	67.73 ± 0.74^{b}
Total lipids	19.05 ± 1.20^{a}	18.99 ± 1.18^{a}	17.67 ± 1.15^{ab}	14.36 ± 2.97^{ab}	15.53 ± 2.90^{ab}	19.12 ± 1.13^{a}	14.64 ± 0.24^{ab}	14.83 ± 1.34^{ab}	13.69 ± 1.41^{b}
Ash	15.15 ± 0.95^{a}	15.57 ± 0.52^{a}	16.46 ± 0.49^{ab}	18.00 ± 1.04^{b}	18.08 ± 0.97^{b}	15.78 ± 0.35^{a}	16.64 ± 0.38^{ab}	17.59 ± 1.32^{b}	18.94 ± 0.27^{b}

to the control group. While a 5% substitution can appear small, it is similar to the 4% reported by Binalshikh-Abubkr & Mohd-Hanafiah (2022) in red hybrid tilapia (*Oreochromis* sp.), while the maximum value of 10% differs from the 20% reported by Prabu et al. (2018) in GIFT tilapia; which could be related to the nutritional composition of the biofloc and the processes to which the biofloc was subjected before its use, in addition to the ability of the cultured species to ingest and digest the microbial biofloc (El-Sayed 2021). Khanjani et al. (2023) reported that biofloc meal had been used directly in diets for penaeid shrimp and tilapia with good growth results when dietary inclusion levels ranged from 4 to 30%.

According to Ekasari et al. (2014), the biofloc is a useful protein source for Nile tilapia since it could meet part of the essential amino acid requirements the species needs for growth. During this study, it was observed that by increasing the percentage of inclusion of biofloc flour (15 and 20%) in the diets, the IWG and SGR of tilapia decreased, possibly due to an imbalance of amino acids in the diets, since the biofloc process to obtain biofloc flour was heterotrophic type dominated by bacteria (Gallardo-Collí et al. 2019). It is known that biofloc has deficiencies in essential amino acids such as arginine, lysine, and methionine (Ju et al. 2008b), which, although integrated into the diets from the fish meal, could present a subclinical deficiency in the tilapia that caused a lower growth. In this study, the protein content of the native biofloc decreased due to the processing used to obtain the biofloc meal, which could also affect the concentration of some essential amino acids (Ekasari et al. 2014).

On the other hand, increasing the percentage of biofloc meals in the diets resulted in harder and less palatable pellets, which is coherent with that reported by Gamboa-Delgado & Márquez-Reyes (2016), who indicated that the excess microbial biomass in aquafeeds could negatively affect the texture and palatability of the feed.

According to Gamboa-Delgado & Márquez-Reyes (2016), the digestibility of an ingredient depends on its chemical composition and the physiological digestion of the species. In this study, the values of ADMD (81-84%) and ACPD (88-90%) in the treatments where 5 and 10% of biofloc meal were added were high, possibly due to the high digestibility of the microbial biomass that was reported (Gamboa-Delgado & Márquez-Reyes 2016), and by the low pH of the tilapia stomach (~1), a physiological adaptation that allows the digestion of the biofloc or extraction of nutrients from cells without breaking the cell wall (Hargreaves 2013). In contrast, the high ash content and the probable presence of trace elements that exert toxic effects could have contributed to the lower digestibility of proteins in diets with higher levels of biofloc (15 and 20%), and some deficiencies regarding essential amino acids may occur, resulting in poor fish growth. The high ash content in biofloc (33-35% ash) could be considered a limiting factor during their use in aquaculture feed since it can affect osmotic homeostasis and increase energy expenditure (Anand et al. 2017). Chen et al. (2018) reported decreased apparent protein and organic material digestibility coefficient while feeding sea cucumber (*Apostichopus japonicus*) with diets containing biofloc meal, which is associated with decreased digestive enzyme activity.

Despite the variations observed in the proximal composition of the Nile tilapia, the results indicate a high quality in the meat, which agrees with what was reported in previous research, where Nile tilapia were fed with "native" biofloc (Pérez-Fuentes et al. 2018).

Currently, the inclusion rate of fishmeal in tilapia diets has decreased due to improvements in feed formulation and manufacturing methods (Tacon & Metian 2008, FAO 2022); despite this, Nile tilapia is among the main consumers of fish meal, since by volume of production it ranks third among freshwater species farmed with aquafeed (Tacon & Metian 2008, Tacon et al. 2011). In this study, two sources of protein in tilapia diets were contrasted as a starting point: fishmeal and biofloc meal; however, to further highlight the potential of biofloc meal as a useful protein ingredient in tilapia feeds, it is necessary to evaluate biofloc meal in diets with few or no fishmeal inclusion and include it in diets based on animal or plant by-products.

CONCLUSION

The results obtained in the parameters of growth, feed efficiency, and digestibility for Nile tilapia suggest that under the experimental conditions of this study, it is possible to replace between 5 to 10% of fishmeal with biofloc meal, thus reducing the need for fishmeal as the main source of protein in the diet. Although the study results can have a practical application, it is also important to identify the biofloc meal and the dietary factors that stimulate and inhibit the growth of Nile tilapia. Given the rise of farming systems with biofloc technology, biofloc processed into meals can be a sustainable ingredient with great potential for use in aquafeeds; nevertheless, it is a priority to continue the search for a sustainable and renewable source of protein-rich ingredients.

ACKNOWLEDGMENTS

This study was funded by Tecnológico Nacional de México (TecNM) with project N°2tp657-3447. AGC was funded by The National Council of Humanities, Science, and Technology (CONAHCYT) of México (scholarship 229713). The authors recognize Andrés Cabrera Muñoz's support during the study.

CREDIT AUTHORSHIP

CIPR, AGC, and MPHV: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Supervision, Project administration, Writing - Original Draft, Writing - Review and Editing.

CONFLICTS OF 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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Received: August 17, 2023; Accepted: January 5, 2024

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