Received: 3 March 2023 | Revised: 15 January 2024 | Accepted: 5 February 2024

DOI: 10.1111/jwas.13062

APPLIED RESEARCH

Journal of the World Aquaculture Society Open Access | WORLD AQUACULTURE Society | WILEY

Use of fermented malted barley by-product as partial feed replacement and carbon source for rearing Nile tilapia (*Oreochromis niloticus*) juveniles

Miguel Cabanillas-Gámez[1] | **Juan Gómez-Cadena**[1] | **Mario A. Galaviz**[2] | **Gustavo Alejandro Rodríguez-Montes de Oca**[3] | **Fernando Figueroa-Saavedra**[1] | **Lus M. López**[2] | **Idaly Trejo-Escamilla**[2] | **Fernando Barreto-Curiel**[2] | **Gilberto López-Valencia**[1]

[1]Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autónoma de Baja California (UABC), Mexicali, Mexico

[2]Facultad de Ciencias Marinas, Universidad Autónoma de Baja California (UABC), Ensenada, Mexico

[3]Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa (UAS), Mazatlán, Mexico

Correspondence
Miguel Cabanillas-Gámez, Universidad Autónoma de Baja California (UABC), Instituto de Investigaciones en Ciencias Veterinarias, km 3.5 Carretera San Felipe, Fraccionamiento Campestre, Mexicali, B.C., Mexico.
Email: miguel.cabanillas@uabc.edu.mx

Mario A. Galaviz, Facultad de Ciencias Marinas, Universidad Autónoma de Baja California (UABC), Ensenada, Mexico.
Email: mgalaviz@uabc.edu.mx

Abstract

The present research evaluates the performance of the Nile tilapia (*Oreochromis niloticus*) (initial weight 11.5 ± 0.07 g) with biofloc technology. For this purpose, spent grains (malted barley) from the brewing industry after aerobic fermentation were used, as both a carbon source (suspended solids) and partial substitute for the diets at 0, 10, 20, 30, and 40% levels (settleable solids). The partial 20% substitution of the diet did not have significant effects on the biological parameters: final body weight, weight gained, average body weight, specific growth rate, daily growth index, condition factor, feed conversion ratio, protein efficiency rate, and viscerosomatic and hepatosomatic indices. However, the dietary substitution showed a significant

effect on the final biofloc chemical composition. Nitrogen compounds, NH_4^+, NO_2^-, and NO_3^-, were adequate for tilapia cultivation in all the treatments. No clear effect of partial dietary substitution was observed on meat quality parameters: pH, color, water holding capacity, and amino acid and fatty acid profile of fillet. The results suggest that a 20% substitution of the diet for malted barley is suitable for satisfactory biological parameters and Nile tilapia fillet quality.

KEYWORDS
biofloc, fermented malted barley, meat quality, performance, tilapia

1 | INTRODUCTION

Currently, aquaculture has been mainly characterized by monocultures, depending on large water volumes and significant amounts of fishmeal for manufacturing balanced feeds, as well as substantial areas of land to obtain high productivity (Boyd et al., 2020; Rodrigues et al., 2019). On the other hand, the inappropriate waste disposal into the environment can have negative impacts, such as soil and water contamination, pathogen transmission, and eutrophication of water bodies affecting sustainability (Boyd et al., 2020; Verdegem, 2013). To ensure sustainability, fish production should meet standards, such as environmental (CO_2, CH_4, energy utilization), economic (production and mobilization of commercial chains), social (good quality and affordable products), and animal welfare aspects (Yacout et al., 2016).

Biofloc technology (BFT) allows a more environmentally friendly culture system that generally occurs in a closed system configuration that minimizes water and nutrient discharge, providing a biosecure and economically sustainable aquaculture production (Browdy et al., 2012). The principle of BFT is to develop aggregates of microorganisms, mainly heterotrophic bacteria, algae, protozoa, and rotifers (Avnimelech, 2009; Monroy-Dosta et al., 2013). Heterotrophic bacteria recycle food waste. For example, organic carbon, ammonia nitrogen, nitrites, nitrates, and phosphates are used as an energy source by oxidizing them into chemical forms and making them usable for algae, fungi, other bacteria, and filter feeders; they keep water quality and increase efficiency in the use of this resource by minimizing turnover rate, and at the same time, a source of complementary food is generated in situ for the cultivated species (Avnimelech, 2009; Correa et al., 2020; Crab et al., 2009; Ekasari et al., 2014; Hargreaves, 2013; Monroy-Dosta et al., 2013; Sgnaulin et al., 2020; Wasielesky et al., 2020). Bacterial populations are stimulated by modifying carbon: nitrogen ratio (C:N ratio) adding external carbon sources and providing sufficient aeration to keep the microbiota and nutrients suspended in the water column (Crab et al., 2012). Other benefits of BFT include the control of pathogenic bacterial populations. Thus, biofloc can play an important role as an immunostimulant in fish and shrimp (Hernández et al., 2019). In addition, nutritional advantages have been observed, such as improvement in the rate of ingestion, digestion, absorption, food conversion, growth, and activity of digestive enzymes, among others, which can optimize crop performance (Hernández et al., 2019).

Several studies on freshwater cultured tilapia with BFT have shown favorable growth results (Aly et al., 2017; Mansour & Esteban, 2017; Muñoz, 2018; Velasco, 2019) even improving feeding efficiency and the antioxidant response of juvenile tilapia (Bañuelos-Vargas et al., 2021). In this sense, biofloc represents an alternative to face one of the main challenges of aquaculture today. For instance, the high cost of food represents more than 50% of the production cost in an aquaculture farm, where commercial feeds traditionally use fishmeal as a protein source (El-Sayed, 1999; FAO, 2009). Thus, one of the research areas that has emerged today is the search for industrial by-products that can be incorporated

into the cultures of aquatic organisms with BFT as a carbon source to generate low-cost in-house protein. Some studies have tested the use of different carbon sources to maintain the appropriate C:N ratio in the BFT: molasses, cassava starch, corn starch, corn flour and molasses (Muñoz, 2018), molasses and wheat flour (Aly et al., 2017), wheat milling by-product, rice bran (Mansour & Esteban, 2017), and brewery by-products (spent grain) (Estévez et al., 2021). Barley flour has been already evaluated as a carbon source for biofloc management with satisfactory results when compared to molasses, corn meal, and starch for growth and nitrogen waste control in tilapia (Khanjani et al., 2021).

Currently, the brewing industry generates large amounts of by-products and waste. Among the most common are grains, used hops, and yeasts, which can be easily recycled and reused (Mussatto et al., 2006). Grains from the brewing industry contain approximately 18%–35.4% protein (Fărcaş et al., 2017). These grains constitute alternative protein sources that have begun to be investigated for the preparation of feeds for aquaculture organisms with favorable results (Estévez et al., 2021), which could help reduce high feeding costs. Large breweries that produce 1000 hectoliters (HL) of beer per day generate up to 40 tons (t) of waste by-products (Thomas & Rahman, 2006). De Macêdo et al. (2023) mentioned that the inclusion of exogenous enzymes can improve the utilization of sorghum distiller dried grains as a dietary component. Other references have already described the feasibility of using barley, either as protein concentrate to replace fishmeal in Caspian brown trout (*Salmo trutta caspius*) (Zaretabar et al., 2021) or directly as brewer's waste, positively replacing up to 25% of fishmeal in experimental diets for juvenile tilapia (Zerai et al., 2008). From these last references, a previous fermentation is inferred as pre-digestion with commercial probiotic bacteria that could increase both carbon availability (suspended solids) and digestibility (SSs) of malted barley by-products. In this case, aerobic fermentation—along with commercial probiotics—serves as exogenous enzyme sources and degrades organic compounds (fiber, etc.) as usable carbon sources besides improving the probiotic–prebiotic–postbiotic complex as naturally occurring feed additives (Marimuthu et al., 2022; Piazzon et al., 2017).

Therefore, the objectives of the present research study are, firstly, to evaluate the effect of using fermented malted barley from the local brewing industry as a partial substitution of commercial feed from 10% to 40% of daily feeding ratio, and secondly, to consider it as an alternative carbon source for the maintenance of BFT culture for feeding Nile tilapia *O. niloticus* juveniles on biological and water quality parameters, blood, proximate composition, amino acid (AA) and fatty acid profile, and meat quality.

2 | MATERIALS AND METHODS

2.1 | Fish

The tilapia *O. niloticus* fry (0.5 ± 0.05 g) were donated by the company Maricultura Del Pacífico S.A. de C.V, Mazatlán Sinaloa, México. The fish were received at the Aquaculture Laboratory of Veterinary Science Research Institute (IICV) and kept in three 1000-L tanks with constant water recirculation and aeration. The organisms were fed a commercial diet (Nutripec-Purina 2.4 mm, 38.4 and 9.1% proteins and lipids, respectively as per our analysis) both during acclimatation (grinded and sieves to 1.0–1.5 mm) and, thereafter, for the feeding experiment at the 2.4 mm size, and the water temperature was maintained at 24°C with 800-watt titanium heaters with temperature controller until the experiment started, and once this temperature was maintained naturally, the heathers were removed from all tanks during the feeding experiment.

2.2 | Biofloc maturation and management conditions

The malted barley was donated by the craft beer company Brew Capital Co, Mexicali, Baja California, México. In the laboratory, the malted barley was packed in 2-kg bags (S.C. Johnson, WI, USA) and stored at −20°C until use. At the

beginning of the experiment, one of the packages was thawed daily, taking the amount necessary for preparing the fermentation used for maturation and maintenance of the biofloc. The nutritional contribution of the malted barley after fermentation was 17.8 and 10.6% of proteins and lipids, respectively.

For the maturation of the biofloc system, (no fish present in experimental units) each of the 15 square fiberglass tanks, measuring 55 × 55 cm on each side and 55 cm high, although the water column was kept at 35 cm to prevent tilapia from jumping upon stocking, giving an approximate volume of 100 L per tank, was inoculated daily with a ferment prepared according to Bañuelos-Vargas et al. (2021), as follows the amount necessary to inoculate each of the biofloc tanks was prepared with a concentration equivalent to 25 g per cubic meter of molasses, wheat bran and ground commercial feed above mentioned and 2.5 g m^{-3} of probiotics (*Saccharomyces cerevisiae*), biodigester (*Bacillus subtilis*), and micronutrients (Micronutrients) from a commercial brand (Bioplanet, Culiacan Sinaloa, México) dissolved in 32 L of water and placed in a reactor provided with sufficient aeration to keep the solids in suspension for 24 h for 30 days prior to the experiment. After that, 2 L of the fermentation was distributed to each of the biofloc tanks. This procedure was carried out daily for 1 month, during which the levels of total ammonia nitrogen (TAN), nitrites, and nitrates were determined as described below every 3 days in all the tanks to monitor the establishment of nitrifying bacterial populations. A 1-hp Sweetwater® triphasic regenerative blower (USA) was used to keep the biofloc and SSs in the water column; the air was distributed to all the tanks with a network of PVC pipes that ended in a circular diffuser hose (an Aerotube® ring 28 centimeters in diameter) placed at the bottom of each tank. Oxygen was maintained above 6 mg L^{-1} all the time of the experiment.

When the nitrite and total ammonia levels stabilized, the juvenile tilapia stocking was carried out: $n = 180$ juveniles (11.57 ± 0.4 g) were stocked in 15 tanks (0.1 m^3) with biofloc culture (12 fish/tank). The stocking density was 120 fish per cubic meter (initial biomass of 1.38 kg m^3). All the organisms were fed a commercial diet (38% protein and 9% lipids) along with the experimental groups in the biofloc system. The experiment consisted of five treatments randomly distributed (three replicates for the treatment) with different substitution percentages of the daily feeding diet based on dry weight for the fermented malted barley (SSs from the reactor) as follows: (1) Bio-Control (Bio-C, 100% commercial diet); (2) Bio-10 (90% commercial diet and 10% fermented malted barley); (3) Bio-20 (80% commercial diet and 20% fermented malted barley); (4) Bio-30 (commercial diet 70% and 30% fermented malted barley); and (5) Bio-40 (commercial diet 60% and 40% fermented malted barley). The chemical composition and AA profile (g/100 g) of the commercial diet and fermented malted barley are shown in Table 1. The food ration was adjusted at 4, 7, 9 and 11 weeks at 5%–3% of fish biomass. After each adjustment in the dietary ration formulated according to biomass, the percentage of commercial diet corresponding to each treatment was subtracted, which was replaced by fermented malted barley until completing 100% of the corresponding daily ration. The wet malted barley and the amount of commercial feed corresponding to each tank were mixed, and both were supplied at the same time to each of the tanks.

For the biofloc management, the amounts of molasses and fermented malted barley needed to maintain the C:N ratio at 10:1 were also adjusted, ensuring that 50% of the carbon required came from each of these two sources and considering both moisture content and daily feed amount per each individual tank. A C:N ratio of 10:1 was based only on the C:N content present in the commercial feed according to Emerenciano et al. (2013). The calculations proposed by Crab et al. (2012) were used, which, according to these authors, 75% of the N contained in the proteins provided in the feed ends up as residual N in the water column. The percentage of C in molasses and wheat was estimated at 28% and 31%, respectively, based on their carbohydrate content, while the estimated percentage was 23.5% in malted barley. Once the biofloc culture had matured and the organisms were stocked, wheat bran was replaced by the beer by-product (malted barley) in the ferment preparation. The adjustment was made as described below, while the bacterial inoculum products were added to a concentration of 2.5 g m^{-3} in the tanks (*Bacillus* plus commercial yeast), with minimal fermentation time of 3 days. After the fermentation process, a filtration process was carried out with a 75-μm mesh to separate the barley from the liquid part. Due to the fermentation time, three reactors were used in an offset manner so that one was ready daily to be used as inoculum. At the end of the

TABLE 1 Chemical composition and amino acid profile (g/100 g) of commercial diet and fermented malted barley.

Amino acid (g/100 g)	Commercial diet	Fermented malted barley
Essential amino acids		
Histidine	1.22 ± 0.01	0.58 ± 0.02
Arginine	3.81 ± 0.02	1.39 ± 0.02
Threonine	1.62 ± 0.01	0.82 ± 0.00
Valine	1.62 ± 0.00	0.90 ± 0.00
Methionine	0.18 ± 0.05	0.17 ± 0.04
Lysine	2.12 ± 0.03	0.88 ± 0.01
Isoleucine	1.56 ± 0.01	0.88 ± 0.00
Leucine	3.61 ± 0.02	1.80 ± 0.02
Phenylalanine	2.09 ± 0.01	1.20 ± 0.01
Subtotal	**17.82 ± 0.07**	**8.60 ± 0.04**
Aspartic acid	2.97 ± 0.03	1.25 ± 0.02
Serine	2.37 ± 0.02	1.10 ± 0.01
Glutamic acid	7.78 ± 0.05	3.70 ± 0.07
Glycine	3.52 ± 0.06	1.15 ± 0.01
Alanine	2.52 ± 0.02	1.20 ± 0.01
Tyrosine	1.40 ± 0.00	0.63 ± 0.01
Subtotal	**20.56 ± 0.07**	**9.02 ± 0.07**
Others		
TAU	Nd	0.22 ± 0.02
Total amino acids	**38.38** ± 0.00	**17.84** ± 0.00
Chemical composition (g/100 g)		
Protein	**38.4**	**17.8**
Lipids	**9.1**	**10.6**
Humidity	**8.1**	**4.5**
Ash	**9.8**	**7.7**
NFE	**34.6**	**59.4**

fermentation process, the malted barley (fermented) was suspended in the water and the corresponding fermentation volume was added to each of the tanks.

The fish were cultured for 98 days in the biofloc system. Experimental procedures related to fish husbandry were approved by the Secretariat of Agriculture, Livestock, Rural Development, Fisheries, and Food (Mexican Official Standard NOM-062-ZOO, 1999).

The food was distributed in two rations at 09:00 h and at 17:00 h. The photoperiod was maintained for 12-h light and 12-h darkness (07:00 and 19:00 h.). Dissolved oxygen (DO mg L^{-1}) was monitored with the dissolved oxygen meter, model 550-A of Yellow Spring Instruments (YSI); temperature (T) and salinity (Sal) were monitored with a conductivity meter model 30 of YSI daily at 09:00 and 17:00-h. Residual nitrogen compounds were monitored every 4 days using commercial colorimetric reagents (Hanna®) for TAN (ammonia NH3/NH_4^+ test solution), nitrites (Nitrite NO_2^- test solution), and nitrates (nitrate NO_3^- test solution), and alkalinity (Alk) was determined indirectly with the GH & KH kit (General & carbonate hardness Kit) (Mars Fishcare North America, Inc. USA), and SS were

checked every 4 days by sedimentation in a separator flask. When the SS exceeded 20 mL L^{-1}, the biofloc sedimentation and removal were allowed at one end of the tank, and when the pH reached 6.5, $NaHCO_3$ was added to rise it to 7.5.

2.3 | Sampling

At the end of the experiment (day 98), the fish were anesthetized with 50 μL L^{-1} clove oil. Then, blood was extracted by cardiac puncture using 1-mL insulin syringe previously filled with EDTA solution as anticoagulant (50 mg mL^{-1} of blood) as described by Blaxhall and Daisley (1973). After that, the fish were euthanized with an overdose of clove oil; three of them were eviscerated to calculate the viscerosomatic index and three more to calculate the hepatosomatic index and proximal content of the viscera and liver, while the muscle was stored for proximal composition. The rest of the fish (six per tank) were immediately stored at −20°C to analyze meat quality, proximal composition, AA, and fatty acid profile of the whole fish.

2.4 | Growth performance

At the end of the test, all the fish were individually weighed, and the biological parameters were calculated with the following formulas:

Final body weight (FBW)

$FBW = FTB/n$

Where

FTB is final total biomass and

N is total number of organisms

Weight gained (WG)

$WG = FBW - IBW$

Where

FBW is final body weight and

IBW is initial body weight

Weight gain percentage (WG %)

$WG\ \% = ((FBW - IBW)/IBW) * 100$

Average body weight (ABW)

$ABW = (FBW + IBW)/2$

Specific growth rate (SGR)

$SGR = Ln\ (FBW) - Ln\ (IBW)/days * 100$

Daily growth index (DGI)

$DGI = ((FBW1/3) - IBW1/3)/days) * 100$

Initial K Condition Factor (Initial K)

$Initial\ K = 100 * (IBW/L\hat{}3)$

Final K Condition Factor (Final K)

$Final\ K = 100 * (FBW/L\hat{}3))$

Where L is length of the body

Feed conversion ratio (FCR)

$FCR = Feed\ consumed/(FBW - IBW)$

Protein efficiency ratio (PER)

PER = (FBW − IBW)/Protein consumed

Hepatosomatic index (HI)

HI = (liver weight/FBW) * 100

Viscerosomatic index (VI)

VI = (viscera weight/body weight) *100

2.5 | Proximate chemical composition

Proximate analyses of the commercial diet fermented and unfermented malted barley, biofloc sediments, muscle, and whole fish were performed using standard methods of the Association of Official Analytical Chemists (AOAC, 2000). The crude protein analysis (CP) (N x 6.25) was performed according to a micro-Kjeldahl method (Labconco Corporation, MO, USA), and the crude lipid (CL) analysis was performed according to a modified Folch method using dichloromethane–methanol (2:1) (Cequier-Sánchez et al., 2008). To obtain moisture (Mo), the samples were weighed in a porcelain tumbler and dried at 105°C to constant weight. Ash was obtained from incineration samples in a muffle (Barnstead/Thermolyne, Thermo Fisher Scientific, MA, USA) at 550°C for 6 h. Nitrogen-free extract (NFE) was calculated using the formula NFE = [100 − (Mo + CP + CL + Ash).

2.6 | Blood analysis

The collected blood was immediately analyzed for hemoglobin (HB) concentration by a standardized procedure using Pointe Scientific, Inc. (MI, USA) kit; the absorbance reading was performed in cuvette using the Hach DR-5000 (UK) spectrophotometer. After that, the hematocrit (Hct) values were determined by the microhematocrit method as proposed by Blaxhall and Daisley (1973). The remaining blood samples were then centrifuged at 12,000 rpm at 4°C for 5 min (Atencio-Garcıa et al., 2007). The plasma collected was immediately frozen and stored at −80°C until total protein (TP), albumin (AL), and glucose (GLU) determinations were performed by a standardized procedure using Pointe Scientific (USA) kits. All the absorbance readings were performed with the microplate reader Multiskan™ GO, Thermo Scientific (MA, USA).

2.7 | Amino acid and fatty acid profile

The analyses of AAs were performed as described by Barreto-Curiel et al. (2019) where 100 mg of biofloc and diet and 20 mg of the tissue samples were previously defatted and dried. Then, they were hydrolyzed to AA with 5 and 1.2 mL of 6 N HCl mixture with 0.06% phenol in 10 mL glass vials. The hydrolysis was carried out by incubating each of the samples at 113°C for 18 h. After the hydrolysis period, the samples were adjusted to a final volume of 100 mL, where they were filtered through 0.45 µm Acrodiscs (P.N. 4426 T). A final volume of 1.5 mL was placed in a previously cleaned, calcined, and amber-colored vial. The samples were refrigerated at −30°C until processing in the high-performance liquid chromatography (HPLC). Derivatization was carried out directly on the Agilent HPLC (Model 1200 Infinity Series, Agilent Technologies, USA). In general, 2.5 µL of phosphate buffer (Part Num. 5061–3339) was taken, followed by 0.5 µL of the sample at a 1:1:1 ratio of OPA:FMOC (orthophthaldehyde: fluorenylmethyloxycarbonyl). Subsequently, they were injected in a continuous sequence into the HPLC. For the separation of the AAS, a reverse-phase C18 Zorbax Eclipse AAA column (4.5 X 150 mm, 3.5 µm, P.N. 963,400–902) was used, with an injection volume of 5 µL. For the run, a sodium phosphate buffer gradient at 40 mM (Sigma-Aldrich, cat.

num. 71500-250g USA.) and a mixture of acetonitrile at 45%, methanol at 45%, and HPLC-grade water at 10% were used at a flow rate of 1 mL/min.

The system is coupled with a fluorescence detector (1260 FLD series, Agilent Technologies, USA) and a DAD detector (1260 DAD-UV, Agilent Technologies, USA). These detectors were configured at two wavelengths, 340/450 nm for excitation/emission in fluorescence and 266/305 nm for excitation/emission, and for the DAD, at 380 nm (OPA) and 262 nm (FMOC).

The calibration curve was performed using a solution of standard AAs (P.N. 061–3330) with concentrations from 50 to 350 picomoles (pmol). Finally, the area under the curve was estimated with the "OpenLAB" program (Agilent Technologies 2000, CA, USA), thus obtaining the percentage of AAs, with respect to the protein content in the samples.

In general, the extraction of fatty acids was carried out by means of a technique described by Folch et al. (1957) and Barreto-Curiel et al. (2017). Small modifications were incorporated, such as adding 0.01% of butylhydroxy-toluene (C15H24O) as an antioxidant solution, working at the lowest possible temperature and making the lipid extraction. To obtain fatty acids, they were separated, identified, and quantified using gas chromatography. An Agilent GC 7820A gas chromatograph was employed, equipped with a split/splitless injector, a flame ionization detector (FID), and an Agilent 122–2361 DB-23 (Agilent Technologies, USA) capillary column measuring 60 m x 0.25 mm with an internal diameter of 15 mm. Calculations were performed using the GC Chemstation Data Analysis software. The initial injection temperature was set at 50°C for 1 min, then raised to 190°C at a rate of 25°C/min, and held for 0 min. Subsequently, it was increased to 230°C at a rate of 6°C/min, and nitrogen (N2) was used as the carrier gas at a flow rate of 0.9 mL/min. Fatty acids were identified by comparison with the retention times of the following standards: 37 Component FAME Mix (Supelco®/Sigma-Aldrich, USA), GLC 87, GLC 96 (Nu-ChekPrep®, MN, USA), RM-2, RM-6, and GLC 90 (Supelco®/Sigma-Aldrich®) and PUFAs from marine oils (PUFA1 and 3, Supelco®/Sigma-Aldrich, USA) were also used as an identification standard. The composition of each fatty acid was calculated according to the corresponding area in the respective chromatogram. C19:0 fatty acid was used as an internal standard. The results are shown as the total percentage of methylated fatty acids.

2.8 | Meat quality analyses

The Delta Trak ISFET pH 101 puncture potentiometer (DeltaTrak, CA, USA) was used to determine pH. Color values (L*, a*, b*, C*, H*) were measured on the cut surface using a MINOLTA CM-2002 spectrophotometer (Minolta Co., Ltd., Japan). A Specular Component Included (SCI), D65 illuminant and a 10° observer were used, where L* is the luminosity index; a* is the red color intensity; b* is the yellow color intensity; and chroma (C*) was calculated as C = (a* 2 + b* 2) 0.5. The water holding capacity (WHC) was performed by means of centrifugation according to the technique of Sutton (1997).

2.9 | Statistical analyses

A randomized design was used in the present study. All data were analyzed for normality (Kolmogorov–Smirnov) and homoscedasticity of variance (Levene's median test). Blood and plasma parameters, growth performance, meat and biofloc composition, AA profile, and meat quality were analyzed by one-way analysis of variance (ANOVA) with the treatment as independent variable. Data are shown as means ± standard error (SE). When significant differences were obtained from the ANOVA, the Holm-Sidak test was used to compare all treatments versus the control group, with a level of significance $p \leq 0.05$ for all statistical tests. When significant differences were observed, correlation

and simple lineal regression analysis were performed to detect the variables with a significant relationship with the treatment. Only the variables with a significant correlation and regression ($p < 0.05$) are presented. All statistical analyses were performed using the computer package SIGMA STAT 3.5 (CA, USA).

3 | RESULTS

3.1 | Water quality

The mean values of the water quality parameters are shown in Table 2. No differences were shown in DO, T, Sal, pH, NH_4^+, NO_2^-, NO_3^-, or SS ($p = 0.257$ to 0.909). Alk was higher ($p = 0.009$) in Bio-30%, and Bio-40% with respect Bio-C. Alk was the only water quality parameter that showed a significant correlation ($p < 0.05$) with the level of diet replacement (Table 11). The regression equation and the coefficient of determination are shown in Figure 2.

3.2 | Growth performance

At the end of the experimental period, significant differences in growth performance and feed efficiency were observed (Table 3). The Bio-10% and Bio-20% diets did not show a significant difference with respect to Bio-C in any of the parameters analyzed. Diet replacement beyond 20% (30%–40%) resulted in a significant decrease in the parameters FBW, WG(g), WG (%), ABW, SGR, DGI, and FCR ($p < 0.016$ to $p = 0.025$), with respect to Bio-C, whereas in HI, a decrease was only observed in Bio-40% ($p = 0.011$), and FCR showed the opposite behavior, increasing in Bio-40% with respect to Bio-C ($p = 0.042$), while none of the treatments showed differences in the condition factor (K), PER or VI versus Bio-C ($p = 0.050$ to $p = 862$). Figure 1 shows the weight in grams at 0, 8, and 14 weeks for all treatments.

Ten of the growth performance parameters analyzed showed a negative correlation ($p < 0.05$) with the diet replacement level, and only VI showed a positive correlation (Table 11). Figure 2 shows the regression equations and the coefficient of determination.

TABLE 2 Water quality parameters of the Nile Tilapia juvenile culture in biofloc systems fed with partial substitution of the diet by fermented malted barley.

	Treatments					
	Bio-C	**Bio-10%**	**Bio-20%**	**Bio-30%**	**Bio-40%**	***p* value**
DO (ppm)	6.34 ± 0.125	6.47 ± 0.120	6.53 ± 0.121	6.42 ± 0.124	6.46 ± 0.124	0.852
T (°C)	24.5 ± 0.27	24.4 ± 0.26	24.2 ± 0.30	24.5 ± 0.26	24.4 ± 0.26	0.909
Sal (‰)	1.31 ± 0.02	1.31 ± 0.02	1.33 ± 0.02	1.3 ± 0.02	1.34 ± 0.02	0.601
pH	7.1 ± 0.12	7.3 ± 0.15	7.4 ± 0.17	7.4 ± 0.19	7.5 ± 0.14	0.434
NH_4^+ (ppm)	0.18 ± 0.08	0.11 ± 0.05	0.01 ± 0.04	0.04 ± 0.04	0.03 ± 0.03	0.301
NO_2^- (ppm)	0.07 ± 0.05	0.07 ± 0.05	0.03 ± 0.03	0.09 ± 0.05	0.09 ± 0.05	0.830
NO_3^- (ppm)	5.1 ± 0.08	5.0 ± 0.00	4.4 ± 0.63a	4.4 ± 0.63	5.0 ± 0.95	0.857
Alk (ppm)	98.1 ± 7.6	127.9 ± 11.1	125.3 ± 9.9	143.3 ± 9.8[a]	156.1 ± 11.8[a]	0.009
SSs (ml L^{-1})	15.8 ± 0.96	14.7 ± 0.91	17.4 ± 1.2	17.3 ± 1.4	14.6 ± 1.2	0.257

Abbreviations: DO, dissolved oxygen, T, temperature; Sal, salinity, pH; NH_4^+, ammonia; NO_2^-, nitrites; NO_3^-, nitrates; Alk, alkalinity; SSs, settleable solids.

[a]Indicates significant differences versus control.

TABLE 3 Growth performance and feed utilization efficiency of tilapia fed experimental diets.

	Treatments					
	Bio-C	**Bio-10%**	**Bio-20%**	**Bio-30%**	**Bio-40%**	***p* value**
IBW (g)	11.6 ± 0.23	11.4 ± 0.24	11.56 ± 0.24	11.57 ± 0.28	12.16 ± 0.24	0.267
FBW(g)	57.03 ± 2.87	51.04 ± 4.70	47.60 ± 0.10	44.39 ± 1.14[a]	43.08 ± 1.15[a]	<0.020
FI (g × fish)	70.91 ± 1.27	68.77 ± 0.51	68.04 ± 0.89	67.79 ± 0.31	67.95 ± 0.66	0.098
WG (g)	45.43 ± 2.91	39.61 ± 4.62	36.04 ± 0.41	32.81 ± 1.25[a]	30.92 ± 1.23[a]	<0.017
WG (%)	391.91 ± 26.9	346.60 ± 39.7	312.72 ± 14.7	283.76 ± 13.7[a]	254.41 ± 11.8[a]	<0.017
ABW (g)	34.31 ± 1.42	31.23 ± 2.40	29.58 ± 0.21	27.98 ± 0.52[a]	27.62 ± 0.54[a]	0.025
SGR	1.62 ± 0.06	1.52 ± 0.09	1.45 ± 0.04	1.37 ± 0.04[a]	1.29 ± 0.03	<0.016
DGI	1.62 ± 0.07	1.48 ± 0.12	1.39 ± 0.03	1.30 ± 0.04[a]	1.23 ± 0.04[a]	<0.016
Initial K	1.66 ± 0.02	1.69 ± 0.03	1.73 ± 0.03	1.71 ± 0.01	1.65 ± 0.02	0.225
Final K	1.88 ± 0.08	1.67 ± 0.15	1.87 ± 0.02	1.81 ± 0.04	1.97 ± 0.15	0.399
FCR	1.60 ± 0.06	1.79 ± 0.23	1.89 ± 0.04	2.07 ± 0.08	2.21 ± 0.11[a]	0.042
PER	1.63 ± 0.06	1.58 ± 0.18	1.55 ± 0.03	1.50 ± 0.06	1.51 ± 0.07	0.862
HI	3.17 ± 0.21	2.74 ± 0.23	3.04 ± 0.25	2.58 ± 0.23	2.15 ± 0.17[a]	0.011
VI	9.31 ± 0.37	9.03 ± 0.27	8.95 ± 0.38	9.67 ± 0.39	10.79 ± 0.30	0.050

Abbreviations: ABW, Average body weight; DGI, daily growth index; FBW, final body weight; FCR, feed conversion ratio; FI, feed intake; Final K, final K condition factor; HI, hepatosomatic index; IBW, initial body weight; Initial K, initial K condition factor; PER, protein efficiency ratio; SGR, Specific growth rate; VI, viscerosomatic index; WG, weight gained; WG%, weight gain percentage.

[a]Indicates significant differences versus control.

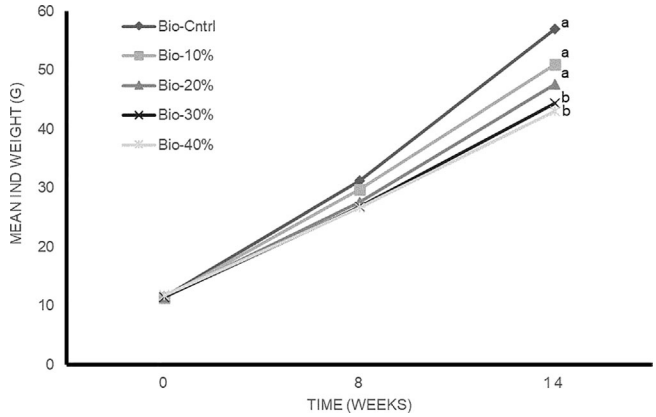

FIGURE 1 Growth in grams of Nile Tilapia juveniles in the biofloc system fed with partial substitution of the diet by fermented malted barley.

3.3 | Proximate composition

The protein and lipid contents in the biofloc, whole fish, and muscle are shown in Table 4. No differences were observed in protein content in muscle ($p = 0.241$) or whole fish ($p = 0.721$). The proteins in the biofloc did not show significant differences in Bio-10% compared to Bio-C; however, the highest percentages of diet replacement (20–40%) did show a decrease in protein content in the biofloc with respect to Bio-C ($p < 0.001$). Lipids increased with

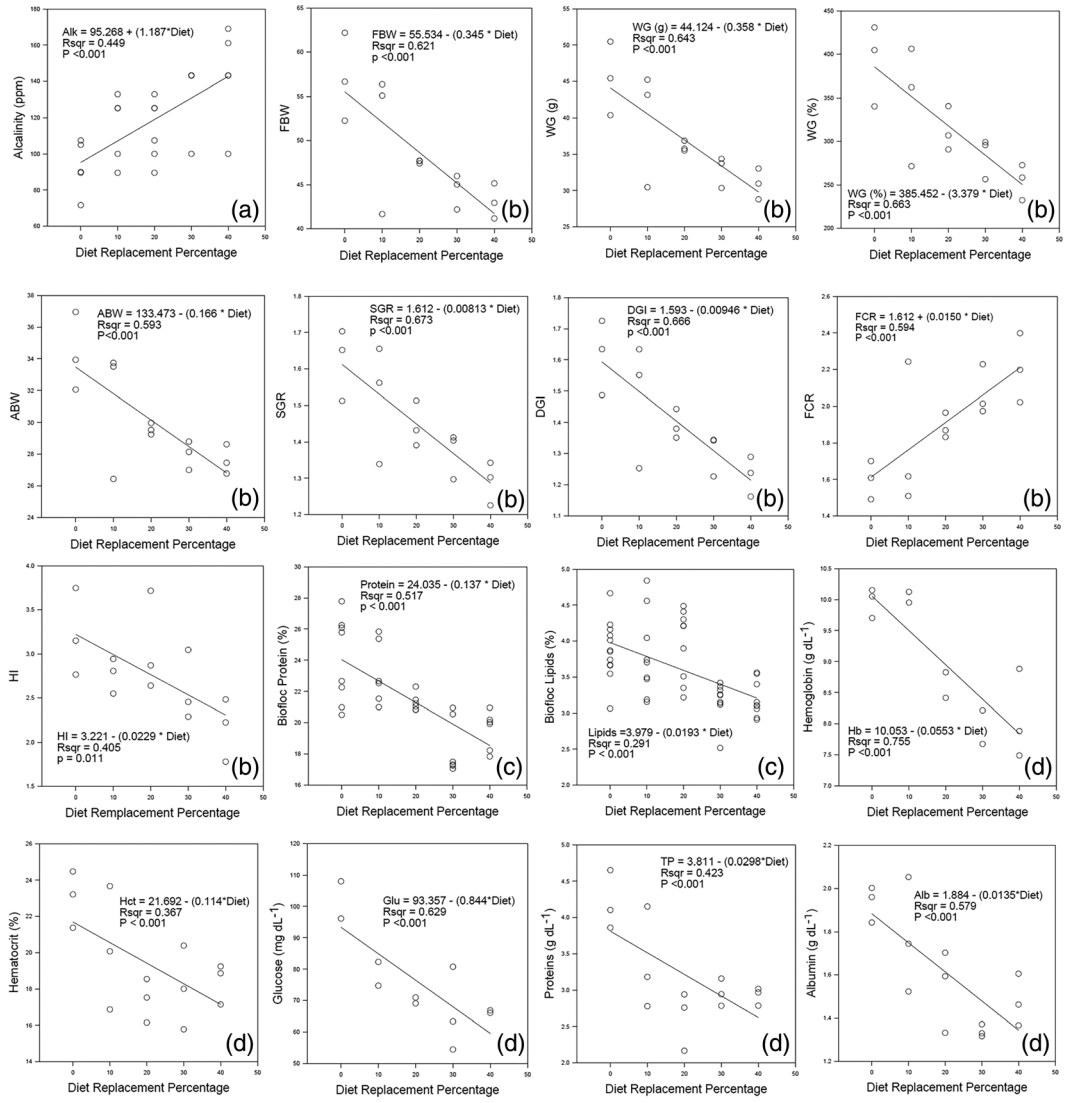

FIGURE 2 Simple linear regression analysis between the variables corresponding to (**a**) water quality; (**b**) growth parameters; (**c**) proximal composition; (**d**) blood and plasma components; (**e**) meat quality and the substitution percentage of the diet; (**f**) amino acids in the biofloc; (**g**) amino acids in the muscle; (**h**) lipids in the biofloc; (**i**) lipids in the muscle and substitution percentage of the diet.

Bio-30% ($p = 0.013$) and Bio-30% and Bio-40% ($p < 0.001$) compared to the control group in the muscle and biofloc, respectively, while lipids in whole fish did not show significant differences ($p = 0.512$).

Only in biofloc, a negative correlation ($p < 0.05$) of protein and lipids was observed with the level of dietary substitution. Figure 2 shows the regression equations and the coefficient of determination.

3.4 | Blood and plasma parameters

The values of blood and plasma parameters are shown in Table 5. No differences in these parameters were observed in Bio-10% compared to Bio-C, except for a decrease in Glu and Glob ($p < 0.001$). However, diet substitution beyond

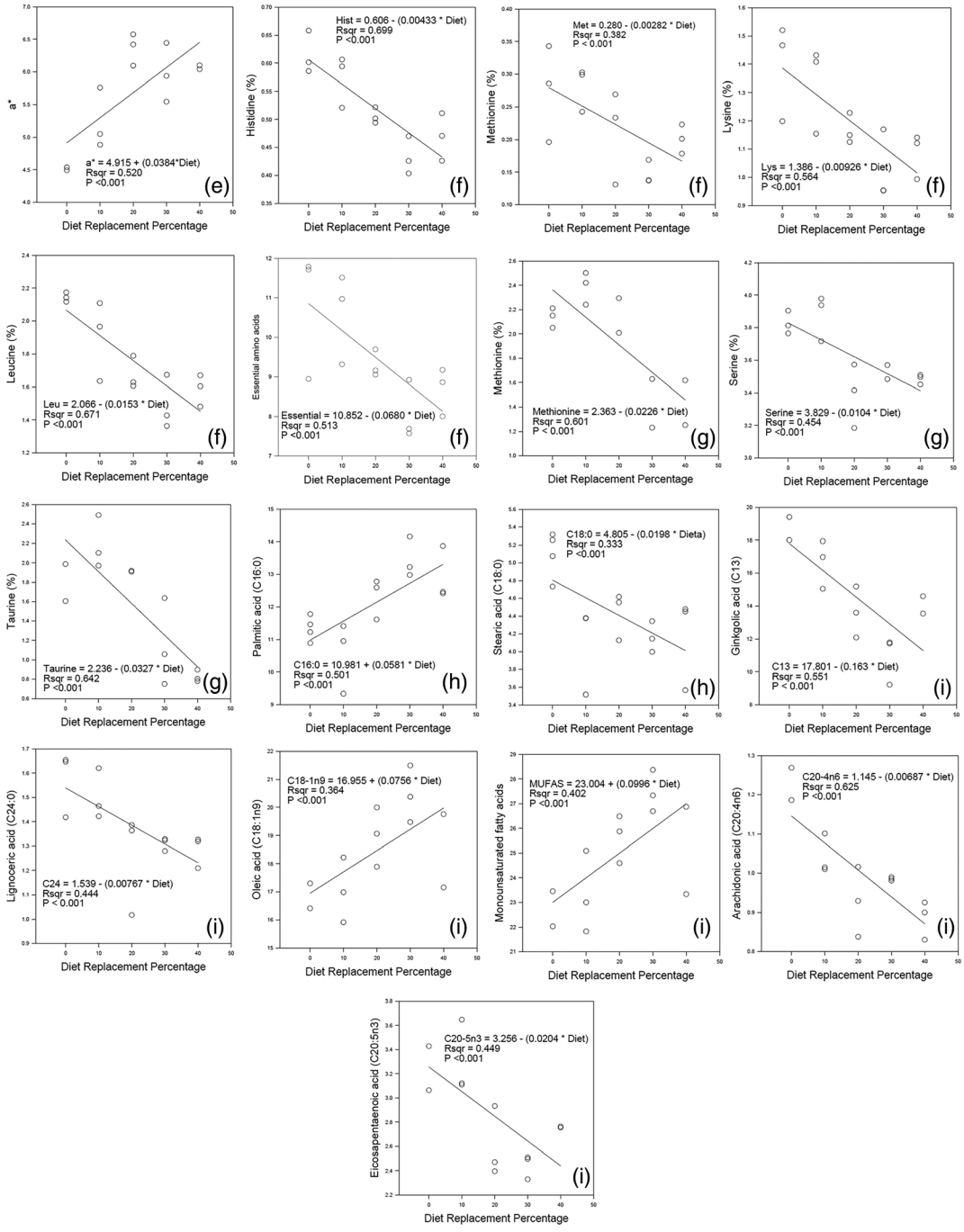

FIGURE 2 (Continued)

10% (20%–40%) decreased all blood and plasma parameters ($p < 0.001$) with respect to Bio-C, except for the Alb:Glob ratio, where no effects of the diet ($p = 0.171$) were observed.

All blood and plasma parameters showed a negative correlation ($p < 0.05$) with the level of dietary replacement (Table 11), except for Glob and Alb:Glob ratio; the regression equations and coefficients are shown in Figure 2.

TABLE 4 Protein and lipid contents (% on dry matter basis) in biofloc, muscle, and whole Nile tilapia juveniles cultured and fed with partial substitution of the diet by fermented malted barley.

		Treatments					
		Bio-C	Bio-10%	Bio-20%	Bio-30%	Bio-40%	*p* value
Muscle	Proteins	79.7 ± 2.2	81.2 ± 1.7	76.1 ± 1.8	77.9 ± 0.7	77.8 ± 0.2	0.241
	Lipids	8.1 ± 0.44	7.1 ± 0.0	9.1 ± 0.7	11.0 ± 0.6[a]	8.8 ± 0.1	0.013
Whole fish	Proteins	48.3 ± 0.1	50.4 ± 3.2	48.9 ± 0.4	48.8 ± 1.7	50.4 ± 0.2	0.721
	Lipids	24.1 ± 3.4	26.8 ± 4.2	22.0 ± 2.7	22.8 ± 1.7	20.1 ± 0.5	0.512
Biofloc	Proteins	24.0 ± 0.9	23.2 ± 0.8	21.3 ± 0.2[a]	18.4 ± 0.7[a]	19.5 ± 0.7[a]	< 0.001
	Lipids	3.9 ± 0.12	3.8 ± 0.20	4.0 ± 0.16	3.2 ± 0.09[a]	3.2 ± 0.08[a]	< 0.001

[a]Indicates significant differences versus control.

TABLE 5 Blood hemoglobin (Hb, g dL^{-1}) and hematocrit (Hct, %) and plasma levels of glucose (Glu, mg dL^{-1}), total proteins (TP, g dL^{-1}), albumin (Alb, g dL^{-1}), and globulins (Glob, g dL^{-1}) of Nile tilapia juveniles cultured in biofloc and fed with partial substitution of the diet by fermented malted barley.

	Treatments					
	Bio-C	Bio-10%	Bio-20%	Bio-30%	Bio-40%	*p* value
Hb	9.9 ± 0.3	10.0 ± 0.4	8.6 ± 0.4[a]	7.9 ± 0.6[a]	8.2 ± 0.4[a]	< 0.001
Hct	22.9 ± 0.8	20.5 ± 1.4	17.6 ± 1.0[a]	17.9 ± 1.0[a]	18.6 ± 0.9[a]	< 0.001
Glu	126.2 ± 8.0	79.1 ± 5.6[a]	70.0 ± 3.8[a]	67.2 ± 4.7[a]	66.4 ± 4.1[a]	< 0.001
TP	4.2 ± 0.2	3.4 ± 0.2	2.6 ± 0.1[a]	3.0 ± 0.2[a]	2.9 ± 0.1[a]	< 0.001
Alb	1.9 ± 0.07	1.8 ± 0.10	1.5 ± 0.06[a]	1.3 ± 0.06[a]	1.5 ± 0.06[a]	< 0.001
Glob	2.6 ± 0.18	1.8 ± 0.14[a]	1.4 ± 0.11[a]	1.8 ± 0.14[a]	1.7 ± 0.10[a]	< 0.001
Alb:Glob	1.01 ± 0.05	1.15 ± 0.05	1.26 ± 0.07	0.93 ± 0.05	1.19 ± 0.17	$p = 0.171$

[a]Indicates significant differences versus control.

3.5 | Meat quality

Table 6 shows the means and standard error of the meat quality parameters pH, color (L*, a*, b*, C*, H*), and WHC. Significant differences ($p < 0.05$) were observed in the treatments with respect to Bio-C. According to data on the meat color variables, similar results were observed between Bio-40% and Bio-C groups, which showed a high bright and pale and less vivid coloration with respect to other treatments, whereas the groups with an intermediate level of diet replacement (Bio-10 to Bio-30%) showed darker meat with a greater number of red pigments and more vivid and less pale color than Bio-C, except for Bio-20% in which the color was paler.

While no clear trend was observed in pH and WHC, only the a* parameter showed a positive correlation ($p < 0.05$) with the level of diet replacement (Table 11). The regression equation and coefficient of determination are shown in Figure 2.

3.6 | Amino acid profile

In biofloc, high percentages of dietary replacement decreased the levels of the essential AAs histidine (His), methionine (Met), lysine (Lys), and leucine (Leu) ($p = 0.043$ to $p < 0.001$); the non-essential AAs serine (Ser) and glycine (Gly) ($p = 0.003$ to 0.012); and the sum of total AAs ($p = 0.030$) with respect to the control diet, while no

TABLE 6 Quality of flesh parameters pH, color values (L*, a*, b*, C*, and H*) and water holding capacity (WHC) of Nile tilapia juveniles cultured in biofloc and fed with partial substitution of the diet by fermented malted barley.

	Treatments					
	Bio-C	Bio-10%	Bio-20%	Bio-30%	Bio-40%	*p* value
pH	6.48 ± 0.02	6.46 ± 0.05	6.50 ± 0.02	6.37 ± 0.04	6.45 ± 0.02	0.046
L*	42.9 ± 1.0	39.1 ± 0.7[a]	35.4 ± 0.8[a]	35.8 ± 0.8[a]	41.1 ± 0.6	< 0.001
a*	3.10 ± 0.64	5.31 ± 0.52[a]	5.35 ± 0.67[a]	6.79 ± 0.68[a]	3.83 ± 0.72	< 0.001
b*	10.8 ± 0.57	12.3 ± 0.47	18.6 ± 0.41[a]	12.1 ± 0.41	11.0 ± 0.42	< 0.001
C*	11.6 ± 0.64	13.4 ± 0.55[a]	19.6 ± 0.49[a]	14.2 ± 0.56[a]	11.6 ± 0.51	< 0.001
H*	76.7 ± 2.9	65.6 ± 2.8[a]	74.7 ± 1.8	61.5 ± 2.2[a]	75.6 ± 2.0	< 0.001
WHC	78.8 ± 0.70	76.3 ± 0.52[a]	77.8 ± 0.56	74.2 ± 0.67[a]	77.3 ± 0.54	< 0.001

[a]Indicates significant differences versus control.

differences were observed in any of the AAs between Bio-10% and Bio-C. Only taurine showed a significant decrease at all levels of dietary replacement with respect to Bio-C ($p < 0.001$) (Table 7).

In the muscle, only the essential AA, Met, and the non-essential AAs Ser and Taurine (Tau) showed a decrease with the highest percentages of dietary replacement with respect to Bio-C. However, Bio-10% did not show any difference with respect to Bio-C in terms of AA content in the muscle (Table 9).

A significant correlation ($p < 0.05$) between AAs and the level of dietary substitution was observed in a greater number of AAs in biofloc, in relation to muscle (Table 11). The equations and coefficient of determination are shown in Figure 2.

3.7 | Fatty acid profile

The only fatty acids that showed differences with any of the percentages of dietary replacement against the Bio-C group were the saturated fatty acids C16:0 (Bio-30 and Bio-40%) and C18:0 (Bio-10 to Bio-40%) and the monounsaturated fatty acid C18:1n9 (Bio-10%) ($p = 0.019$ to $p = 0.003$) (Table 8). However, in the muscle, the fatty acids that showed a significant difference—due to the level of substitution of the diet with respect to Bio-C—were the saturated fatty acids C13:0 (Bio-20 to Bio-40%) and C24:0 (Bio-20%); the monounsaturated fatty acids C16:1n7 (Bio-20 and Bio-30%) and C18:1n9 (Bio-30%); and the polyunsaturated fatty acids C20:4n6 (Bio-10 to Bio-30%) and C20:5n3 (Bio-30%) ($p = 0.035$ to $p < 0.001$) (Table 10).

A significant correlation ($p < 0.05$) between fatty acids and the level of dietary substitution was observed in a greater number of fatty acids in muscle, in relation to biofloc (Table 11). The equations and coefficient of determination are shown in Figure 2.

4 | DISCUSSION

Cultivation in biofloc is considered a symbiotic process that, in addition to aquatic animals in culture, is characterized by the presence of aggregates of heterotrophic bacteria, algae, protozoa, and other microbial species. They are united in a matrix together with particular organic material suspended in culture water, where bacteria perform the function of improving water quality, waste treatment, and, in some cases, disease resistance (El-Sayed, 2019). In biofloc, the addition of external sources of organic carbon allows an increase in the carbon: nitrogen (C:N) ratio in the water column, which favors the rapid development of heterotrophic bacteria that immobilize dissolved inorganic

TABLE 7 Amino acid profile (g/100 g) of biofloc of tilapia culture using malted barley as C source and partial dietary substitute.

	Treatments					
Amino acid	**Bio-C**	**Bio-10%**	**Bio-20%**	**Bio-30%**	**Bio-40%**	***p* value**
Essential amino acids						
Histidine	0.62 ± 0.02	0.57 ± 0.03	0.51 ± 0.01[a]	0.43 ± 0.02[a]	0.47 ± 0.02[a]	< 0.001
Arginine	1.61 ± 0.31	1.65 ± 0.11	1.50 ± 0.07	1.13 ± 0.03	1.25 ± 0.09	0.155
Threonine	1.19 ± 0.11	1.26 ± 0.09	1.08 ± 0.03	1.00 ± 0.06	1.05 ± 0.03	0.136
Valine	1.46 ± 0.15	1.47 ± 0.10	1.30 ± 0.04	1.19 ± 0.07	1.27 ± 0.05	0.214
Methionine	0.28 ± 0.04	0.28 ± 0.02	0.21 ± 0.04	0.15 ± 0.01[a]	0.20 ± 0.01	0.043
Lysine	1.40 ± 0.10	1.33 ± 0.09	1.17 ± 0.03	1.03 ± 0.07[a]	1.09 ± 0.05[a]	0.020
Isoleucine	0.84 ± 0.11	0.88 ± 0.07	0.76 ± 0.04	0.70 ± 0.05	0.75 ± 0.03	0.359
Leucine	2.15 ± 0.02	1.90 ± 0.14	1.67 ± 0.06[a]	1.49 ± 0.09[a]	1.59 ± 0.06[a]	0.002
Phenylalanine	1.28 ± 0.15	1.24 ± 0.09	1.10 ± 0.04	0.94 ± 0.06	1.04 ± 0.05	0.098
Subtotal	**10.81 ± 0.94**	**10.60 ± 0.66**	**9.30 ± 0.20**	**8.06 ± 0.43[a]**	**8.68 ± 0.35**	**0.028**
Non-essential amino acids						
Aspartic acid	2.36 ± 0.23	2.20 ± 0.26	2.03 ± 0.08	1.89 ± 0.12	2.00 ± 0.07	0.304
Serine	1.62 ± 0.07	1.56 ± 0.08	1.40 ± 0.01	1.20 ± 0.09[a]	1.21 ± 0.05[a]	0.003
Glutamic acid	3.16 ± 0.23	3.14 ± 0.23	2.73 ± 0.04	2.50 ± 0.18	2.55 ± 0.10	0.046
Glycine	2.77 ± 0.21	2.67 ± 0.11	2.49 ± 0.02	2.06 ± 0.15[a]	2.14 ± 0.10[a]	0.012
Alanine	2.37 ± 0.25	2.35 ± 0.09	2.13 ± 0.01	1.82 ± 0.15	1.89 ± 0.07	0.057
Tyrosine	0.93 ± 0.08	0.91 ± 0.08	0.82 ± 0.03	0.70 ± 0.07	0.72 ± 0.05	0.092
Subtotal	13.20 ± 1.03	12.15 ± 0.81	11.60 ± 0.12	10.11 ± 0.74	10.52 ± 0.44	0.063
Others						
Taurine	0.51 ± 0.02	0.43 ± 0.04[a]	0.37 ± 0.01[a]	0.30 ± 0.01[a]	0.34 ± 0.01[a]	< 0.001
Total	24.53 ± 1.93	23.17 ± 1.27	21.27 ± 0.32	18.47 ± 1.17[a]	19.53 ± 0.79[a]	0.030

[a]Indicates significant differences versus control.

nitrogen (Oliveira et al., 2022). Bacteria assimilate residual nitrogen from fish waste and uneaten food scraps into a protein-rich microbial biofilm that is easily accessible to fish, improving growth rate (Haraz et al., 2023).

Because of the benefits of tilapia *O. niloticus* biofloc rearing, some studies have tested different sources of C (molasses, cassava starch, corn starch, rice bran, corn flour, wheat flour, wheat milling, and brewery by-products) to maintain the appropriate C:N ratio (Aly et al., 2017; Estévez et al., 2021; Mansour & Esteban, 2017; Muñoz, 2018).

On the other hand, protein predominates as the most important component in fish diet formulation, with fishmeal being the preferred protein source for many aquaculture species due to its balance of AAs, vitamin content, palatability, and energy content (Martin & Pagare, 2016); however, the high costs of fishmeal could compromise aquaculture sustainability (Boyd et al., 2020; Rodrigues et al., 2019). Thus, the search for alternative protein sources constitutes a topic of great importance, and the use of industrial by-products is of particular interest. Considering that brewery by-products are made in large quantities, they can be potential alternatives to reduce the use of plant proteins or fish/animal by-products (trimmings) and increase the sustainability of both sectors, brewery industry, and aquaculture alike (Estévez et al., 2021). Therefore, the results in the present study evaluate the performance and meat quality of tilapia *O. niloticus* in biofloc culture using malted barley (brewing by-product) as carbon source

TABLE 8 Fatty acid profile (% of methylated fatty acids) of biofloc culture of tilapia juvenile using malted barley as C source and partial dietary substitute.

	ID Fatty acids	Bio-C	Bio-10%	Bio-20%	Bio-30%	Bio-40%	*p* Value
Caprylic acid	C8:0	0.33 ± 0.21	0.33 ± 0.33	0.21 ± 0.21	0.28 ± 0.28	0.20 ± 0.20	0.992
Capric acid	C10:0	0.39 ± 0.23	0.41 ± 0.41	0.28 ± 0.28	0.26 ± 0.26	nd	0.976
Undecylic acid	C11:0	0.54 ± 0.19	0.46 ± 0.24	0.34 ± 0.34	nd	0.26 ± 0.26	0.848
Ginkgolic acid	C13:0	41.15 ± 2.25	41.67 ± 2.36	39.75 ± 1.72	38.53 ± 1.36	40.34 ± 1.04	0.807
Myristic acid	C14:0	1.18 ± 0.09	1.07 ± 0.23	1.10 ± 0.03	1.07 ± 0.06	1.05 ± 0.05	0.92
Pentadecylic acid	C15:0	1.01 ± 0.08	1.26 ± 0.09	0.85 ± 0.12	0.99 ± 0.01	0.95 ± 0.12	0.103
Palmitic acid	C16:0	11.34 ± 0.19	10.56 ± 0.63	12.33 ± 0.36	13.46 ± 0.36[a]	12.92 ± 0.48[a]	0.003
Heptadecanoic acid	C17:0	Nd	0.21 ± 0.21	0.435 ± 0.18	nd	nd	0.618
Stearic acid	C18:0	5.10 ± 0.13	4.09 ± 0.19[a]	4.43 ± 0.15[a]	4.16 ± 0.10[a]	4.16 ± 0.30[a]	0.017
Arachidic acid	C20:0	nd	nd	0.20 ± 0.20	0.25 ± 0.25	nd	0.875
Lignoceric acid	C24:0	0.90 ± 0.30	0.92 ± 0.05	0.94 ± 0.12	1.06 ± 0.07	0.92 ± 0.19	0.978
Saturated fatty acids	**ΣSFA**	**61.93 ± 1.13**	**60.97 ± 2.07**	**60.77 ± 1.10**	**60.07 ± 1.36**	**60.80 ± 1.27**	**0.903**
Tetradecanoic acid	C14:1	2.33 ± 0.46	2.73 ± 0.94	2.06 ± 0.06	2.06 ± 0.08	2.01 ± 0.11	0.814
Pentadecanoic acid	C15:1	0.98 ± 0.09	0.94 ± 0.10	0.80 ± 0.08	0.72 ± 0.02	0.75 ± 0.10	0.185
Palmitoleic acid	C16:1n7	2.98 ± 0.31	3.36 ± 0.13	2.61 ± 0.30	2.65 ± 0.24	2.85 ± 0.10	0.294
Oleic acid	C18:1n9	8.01 ± 0.40	6.32 ± 0.25[a]	7.51 ± 0.14	7.78 ± 0.30	7.28 ± 0.21	0.019
Cis-Vaccenic acid	C18:1n7	6.40 ± 0.58	7.04 ± 0.84	6.52 ± 0.34	6.53 ± 0.35	6.77 ± 0.59	0.934
Monounsaturated fatty acids	**ΣMUFAS**	**20.71 ± 0.99**	**20.39 ± 2.05**	**19.50 ± 0.67**	**19.73 ± 0.32**	**19.65 ± 0.76**	**0.912**
Linoleic acid	C18:2n6	9.52 ± 1.39	8.41 ± 0.57	10.39 ± 0.46	11.2 ± 1.83	10.65 ± 1.90	0.678
Octadecatrienoic acid	C18:3n4	0.83 ± 0.29	1.09 ± 0.16	1.04 ± 0.02	1.00 ± 0.08	1.00 ± 0.12	0.871
α-Linolenic acid	C18:3n3	1.31 ± 0.11	0.91 ± 0.06	1.05 ± 0.03	0.98 ± 0.12	1.03 ± 0.13	0.091
Stearidonic acid	C18:4n3	Nd	Nd	Nd	0.2 ± 0.24	Nd	-
Eicosatrienoic acid	C20:3n3	1.42 ± 0.19	1.94 ± 0.25	1.52 ± 0.03	1.24 ± 0.23	1.58 ± 0.25	0.269
Eicosatrienoic acid	C20:4n3	Nd	Nd	Nd	Nd	0.3 ± 0.19	-
Eicosapentaenoic acid	C20:5n3	Nd	Nd	0.40 ± 0.21	0.90 ± 0.36	0.40 ± 0.20	0.367
Docosapentaenoic acid	C22:5n3	Nd	Nd	0.50 ± 0.29	0.35 ± 0.18	0.40 ± 0.20	0.894
Polyunsaturated fatty acids	**ΣPUFAS**	**13.08 ± 1.17**	**12.34 ± 0.43**	**14.91 ± 0.83**	**15.87 ± 2.16**	**15.37 ± 1.9**	**0.392**
Unidentified	NID	4.29 ± 1.0	6.30 ± 0.17	4.83 ± 0.62	4.35 ± 0.58	4.18 ± 0.39	0.268
TOTAL	Σtotal	100.0	100.0	100.0	100.0	100.0	

[a]Indicates significant differences versus control.

and vegetable protein, partially replacing the diet as a strategy to integrate an industrial by-product to reduce both commercial feed and water utilization.

In general, water quality parameters (T, DO, pH, Alk, NH_4^+, NO_2^-, NO_3^-) remained within the acceptable ranges for tilapia farming in biofloc (Avnimelech, 2011) in all treatments. The water temperature was within the acceptable ranges for the normal growth of tilapia (20–35°C) suggested by El-Sayed (2006), optimum temperature estimated from 25 to 30°C (Crab et al., 2009), and ideal temperature for stable biofloc culture (20–25°C) according to De Schryver et al. (2008).

TABLE 9 Amino acid muscle profile (g/100 g) of Nile tilapia juveniles cultured in biofloc and fed with partial substitution of the diet by fermented malted barley.

	Treatments					
Amino acid	**Bio-C**	**Bio-10%**	**Bio-20%**	**Bio-30%**	**Bio-40%**	***p* value**
Essential amino acids						
Histidine	2.10 ± 0.01	2.25 ± 0.06	2.30 ± 0.10	2.19 ± 0.05	2.03 ± 0.02	0.039
Arginine	6.20 ± 0.13	6.08 ± 0.20	5.58 ± 0.23	6.00 ± 0.30	5.41 ± 0.04	0.053
Threonine	4.12 ± 0.10	4.13 ± 0.07	3.91 ± 0.11	3.81 ± 0.18	3.96 ± 0.02	0.246
Valine	4.04 ± 0.23	3.91 ± 0.11	3.86 ± 0.13	4.00 ± 0.04	3.93 ± 0.06	0.881
Methionine	2.14 ± 0.05	2.39 ± 0.08	2.15 ± 0.14	1.43 ± 0.20[a]	1.44 ± 0.18[a]	0.002
Lysine	6.73 ± 0.13	6.65 ± 0.15	6.21 ± 0.05[a]	6.91 ± 0.09	7.06 ± 0.07	0.003
Isoleucine	3.94 ± 0.11	4.04 ± 0.06	3.88 ± 0.09	3.83 ± 0.11	3.95 ± 0.03	0.485
Leucine	6.88 ± 0.08	6.68 ± 0.17	6.44 ± 0.22	7.31 ± 0.45	6.95 ± 0.06	0.245
Phenylalanine	3.47 ± 0.05	3.67 ± 0.07	3.63 ± 0.09	3.90 ± 0.17	3.81 ± 0.05	0.081
Subtotal	**39.31 ± 0.20**	**40.13 ± 0.78**	**37.69 ± 0.59**	**38.67 ± 0.63**	**38.80 ± 0.15**	**0.100**
Non-essential amino acids						
Aspartic acid	8.54 ± 0.18	8.46 ± 0.13	8.17 ± 0.18	8.52 ± 0.11	8.62 ± 0.07	0.286
Serine	3.83 ± 0.04	3.88 ± 0.08	3.40 ± 0.11[a]	3.53 ± 0.04[a]	3.49 ± 0.02[a]	0.003
Glutamic acid	13.06 ± 0.25	13.24 ± 0.21	12.76 ± 0.35	13.34 ± 0.25	13.69 ± 0.10	0.178
Glycine	4.58 ± 0.17	4.41 ± 0.16	4.28 ± 0.17	4.71 ± 0.06	4.29 ± 0.19	0.287
Alanine	5.48 ± 0.10	5.63 ± 0.18	5.15 ± 0.28	5.33 ± 0.16	4.93 ± 0.04	0.106
Tyrosine	3.44 ± 0.23	3.27 ± 0.06	3.10 ± 0.09	3.31 ± 0.18	3.14 ± 0.03	0.480
Subtotal	**38.92 ± 0.64**	**38.89 ± 0.77**	**36.86 ± 1.09**	**38.53 ± 0.42**	**38.15 ± 0.24**	**0.313**
Others						
TAU	1.80 ± 0.19	2.19 ± 0.16	1.91 ± 0.01	1.15 ± 0.26	0.83 ± 0.04[a]	0.002
Total	**80.96 ± 1.53**	**81.21 ± 1.70**	**76.11 ± 1.81**	**77.98 ± 0.71**	**77.78 ± 0.24**	**0.094**

[a]Indicates significant differences versus control.

Salinity increased during cultivation from 0.8 to 1.3 ppt, which may be related to settled solids and zero-water exchange in the biofloc systems. Moreover, the salinity difference from 0.8 to 1.3 ppt in this experiment did not represent a stress factor for Tilapia, a species with the ability to tolerate a wide range of water salinity (El-Sayed, 2006).

Lower Alk levels were observed in Bio-C compared to Bio-30% and Bio-40%, possibly related to a better biofloc performance in Bio-C. The pH and Alk had to be corrected several times by the addition of $NaHCO_3$ to raise Alk to the range recommended (Oviedo et al., 2013) for crop species (50–150 mg L^{-1}). A decrease in Alk may be due both to the assimilation processes by heterotrophic bacteria and nitrification by chemoautotrophic bacteria (Brú-Cordero et al., 2017). Thus, low pH and Alk could be indicators of a good assimilation and nitrification processes. Nitrification requires 8 mg of HCO_3^- to oxidize 1-mg of TAN (Gujer & Jenkins, 1974; Sharma & Ahlert, 1977). According to Ebeling et al. (2006), heterotrophic and autotrophic processes require 7.14 g of $CaCO_3$ per gram of ammoniacal nitrogen reduced to nitrate to maintain adequate alkalinity levels. Thus, lower pH and alk values indicate higher bacterial metabolism in biofloc. Although the decrease in pH in Bio-C was not significant in the present study, the work carried out in the laboratory demonstrates that cultures with high bacterial activity (biofloc) tend to decrease the pH level more quickly than crops in recirculation aquaculture systems (RASs), where the bacterial activity is much lower (data not reported). According to our data, other authors have observed a decrease in pH values due to bacterial activity in biofloc cultures (Kishawy et al., 2020). Low oscillation in the ammonia levels observed over the experiment

TABLE 10 Fatty acid profile (% of methylated fatty acids) of muscle of Nile tilapia juveniles fed with partial replacement of the diet by fermented malted barley.

Fatty acids	ID Fatty acids	Bio-C	Bio-10%	Bio-20%	Bio-30%	Bio-40%	*p* value
Ginkgolic acid	C13:0	18.71 ± 0.70	16.65 ± 0.85	13.62 ± 0.90[a]	10.90 ± 0.85[a]	14.07 ± 0.53[a]	0.002
Myristic acid	C14:0	1.32 ± 0.22	1.25 ± 0.06	1.62 ± 0.05	1.66 ± 0.08	1.33 ± 0.10	0.106
Palmitic acid	C16:0	15.67 ± 0.54	14.98 ± 0.17	15.63 ± 0.18	16.08 ± 0.21	15.54 ± 0.44	0.330
Heptadecanoic acid	C17:0	Nd	0.30 ± 0.02	Nd	Nd	Nd	0.653
Stearic acid	C18:0	6.83 ± 0.37	6.39 ± 0.16	6.30 ± 0.25	6.15 ± 0.18	6.52 ± 0.20	0.388
Eicosanoic acid	C21:0	Nd	Nd	0.089 ± 0.09	0.092 ± 0.09	0.089 ± 0.09	1.000
Lignoceric acid	C24:0	1.57 ± 0.07	1.50 ± 0.06	1.26 ± 0.12[a]	1.31 ± 0.02	1.29 ± 0.04	0.035
Saturated fatty acids	**ΣSFA**	**42.07 ± 1.59**	**41.07 ± 0.66**	**38.51 ± 1.09**	**36.20 ± 0.58[a]**	**40.06 ± 0.71**	**0.015**
Pentadecanoic acid	C15:1	0.47 ± 0.01	0.49 ± 0.03	0.44 ± 0.01	0.41 ± 0.01	0.47 ± 0.04	0.472
Palmitoleic acid	C16:1n7	1.70 ± 0.25	1.84 ± 0.11	2.28 ± 0.08[a]	2.55 ± 0.05[a]	2.01 ± 0.11	0.009
Oleic acid	C18:1n9	16.86 ± 0.44	17.04 ± 0.67	19.00 ± 0.61	20.45 ± 0.58[a]	18.46 ± 1.3	0.035
cis-Vaccenic acid	C18:1n7	2.89 ± 0.10	2.88 ± 0.10	2.91 ± 0.07	2.97 ± 0.05	3.00 ± 0.13	0.853
Gadoleic acid	C20:1n9	1.13 ± 0.07	1.05 ± 0.07	1.07 ± 0.01	1.12 ± 0.03	1.09 ± 0.09	0.875
Monounsaturated fatty acids	**ΣMUFAS**	**22.74 ± 0.71**	**23.31 ± 0.96**	**25.65 ± 0.56**	**27.46 ± 0.49[a]**	**25.1 ± 1.77**	**0.029**
Palmitoleic acid	C16:2n4	Nd	Nd	0.33 ± 0.00	0.35 ± 0.02	0.33 ± 0.01	0.251
Linoleic acid	C18:2n6	15.86 ± 0.61	15.65 ± 0.37	18.42 ± 1.06	18.97 ± 0.38	15.27 ± 0.12	0.010
γ-Linolenic acid	C18:3n6	0.93 ± 0.05	0.87 ± 0.02	0.84 ± 0.04	0.98 ± 0.02	0.82 ± 0.02	0.017
α-linolenic acid	C18:3n3	1.32 ± 0.06	1.32 ± 0.03	1.47 ± 0.08	1.51 ± 0.04	1.23 ± 0.04	0.025
Dihomo-γ-linolenic acid	C20:3n6	1.30 ± 0.07	1.26 ± 0.03	1.25 ± 0.02	1.31 ± 0.02	1.25 ± 0.02	0.644
Arachidonic acid	C20:4n6	1.23 ± 0.04	1.04 ± 0.03[a]	0.93 ± 0.05[a]	1.00 ± 0.00[a]	0.90 ± 0.03	<0.001
Eicosapentaenoic acid	C20:5n3	3.25 ± 0.18	3.29 ± 0.18	2.60 ± 0.17	2.45 ± 0.06[a]	2.76 ± 0.00	0.009
Docosapentaenoic acid	C22:5n3	1.11 ± 0.03	1.27 ± 0.04	1.24 ± 0.07	1.25 ± 0.10	1.35 ± 0.03	0.164
Docosahexaenoic acid	C22:6n3	4.53 ± 0.44	4.57 ± 0.16	3.83 ± 0.29	3.67 ± 0.22	4.80 ± 0.46	0.143
Polyunsaturated fatty acids	**ΣPUFAS**	**28.35 ± 0.64**	**29.26 ± 0.34**	**31.0 ± 0.77**	**31.47 ± 0.28**	**30.10 ± 1.21**	**0.072**
Unidentified	**NID**	**6.0 ± 0.03**	**6.37 ± 0.21**	**4.90 ± 0.39**	**4.88 ± 0.16**	**5.94 ± 0.47**	**0.025**
Total	**Σtotal**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	

Abbreviation: Nd, not determined.
[a]Indicates significant differences versus control.

demonstrates the advantage of the biofloc system since the microorganisms found in the water can control this toxic compound (Kishida et al., 2008). Low nitrite levels acceptable for fish culture (Lucas et al., 2019) and no significant differences of this parameter were observed in all the treatments, indicating that nitrifying bacteria were correctly established in the biofloc system. The low levels of TAN observed during the culture indicate a good functioning of the biofloc (Avnimelech, 2011; Velasco, 2019).

Blood analysis in fish constitutes an invaluable tool to diagnose and determine health and nutritional status of the organisms (Atencio-Garcıa et al., 2007). Factors such as fish species, developmental stage, sex, stress, environmental factors, seasons of the year, availability, and food quality can influence blood and plasma parameters in fish

TABLE 11 Correlation between hematological variables, growth parameters, chemical composition, percentage of amino acids and fatty acids, and diet. Only significant correlations are shown ($p < 0.05$).

	Water quality	Growth parameters							
	Alk	FBW	WG (g)	WG (%)	ABW	SGR	DGI	FCR	HI
Diet	r = 0.670	r = −0.788	r = −0.802	r = −0.814	r = −0.770	r = −0.820	r = −0.816	r = 0.771	r = −0.637
	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.011$

	Biofloc chemical composition		Blood components		Plasma parameters			Meat quality
	Protein	Lipids	Hb	Hct	Glu	TP	Alb	a*
Diet	r = − 0.719	r = − 0.539	r = −0.869	r = −0.606	r = −0.793	r = −0.651	r = −0.761	r = −0.721
	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.017$	$p = 0.004$	$p = 0.008$	$p < 0.001$	$p = 0.005$

	Biofloc amino acids										Biofloc fatty acids	
	Histidine	Methionine	Lysine	Leucine	∑Essent. aa.	Serine	Glutamic Acid	Glycine	Taurine	Total aa.	C16:0	C18:0
Diet	r = −0.836	r = −0.618	r = −0.751	r = −0.819	r = −0.716	r = −0.845	r = −0.704	r = −0.779	r = −0.830	r = −0.736	r = 0.708	r = −0.577
	$p < 0.001$	$p = 0.014$	$p = 0.001$	$p < 0.001$	$p = 0.003$	$p < 0.001$	$p = 0.003$	$p < 0.001$	$p < 0.001$	$p = 0.002$	$p = 0.002$	$p = 0.020$

	Muscle amino acids			Muscle fatty acids					
	Methionine	serine	Taurine	C13:0	C24:0	C18:1n9	∑MUFA	C20:4n6	C20:5n3
Diet	r = −0.775	r = −0.673	r = −0.801	r = −0.742	r = −0.667	r = 0.603	r = 0.634	r = −0.790	r = −0.670
	$p = 0.003$	$p = 0.008$	$p < 0.001$	$p = 0.004$	$p = 0.007$	$p = 0.029$	$p = 0.020$	$p < 0.001$	$p = 0.012$

(Buenaño, 2010; Huss, 1988; Meraj et al., 2016; Rey-Vásquez & Guerrero, 2007; Sáez et al., 2018; Sánchez et al., 2017; Satheeshkumar et al., 2012; Yilmaz, 2015).

In the case of Hct, the Bio-C results were similar to those reported by Akinrotimi et al. (2010) but lower than those of other authors (Hahn-Von-Hessberg et al., 2011; Hrubec et al., 2000; Mansour & Esteban, 2017; Muñoz, 2018) in tilapia. Likewise, Hb in Bio-C was according to previous reports (Akinrotimi et al., 2010; Hahn-Von-Hessberg et al., 2011; Hrubec et al., 2000). The results of TP, Alb, Glob, and Gluc were very similar to previous reports (Hrubec et al., 2000; Mansour & Esteban, 2017). The similarity between the values obtained in the present research study and those reported by other authors indicate that fish have adapted correctly to the biofloc system culture.

However, when the mean values of blood and plasma parameters were compared between Bio-C and any dietary substitution levels, a significant difference was observed since increasing the percentage of fermented malted barley (Bio-20 to Bio-40%) generates a decrease in all blood and plasma parameters, except for the Alb:Glob ratio. The decrease in hemoglobin and hematocrit indicating a trend in anemia and consequently hypoxemia and lower TP means a lower nutritional contribution. The decrease in GLU as the substitution level increased is relevant since glucose is essential for energy generation and, consequently, the growth rate is compromised.

In general, hematological and plasma values have been related to the nutritional and health status of fish. For example, higher values of Hct and Hb have been related to better efficiency in oxygen transport required for metabolism in tissues (Aguirre-Guzman et al., 2016); a high level of TP and Alb has been associated with a strong immune system of the organisms (Adineh et al., 2019), while glucose can present alterations due to a series of factors, among which liver glycogen reserves and nutritional status stand out (Thrall et al., 2015). Therefore, lower blood and plasma parameters were evident as the level of dietary replacement by fermented malted barley increased, indicating that the nutritional status of fish may be compromised if the percentage of dietary replacement exceeds its optimal levels.

However, the results in the present study show that no significant difference exists between Bio-10% and the Bio-C treatments in most of the hematological variables evaluated, except for Glu and Glob, which suggests that the nutritional status of the fish was not strongly affected at this level of diet replacement.

Similar growth parameters were shown in the biofloc culture using a combination of fermented malted barley and molasses as C sources, with respect to previous reports using traditional sources. These data supported by the water quality parameters, which were maintained in the appropriate ranges, indicate the establishment of adequate bacterial populations in the biofloc culture that were capable of assimilating the waste generated in the culture system. WG (g) and FCR were similar; however, SGR and DGI were higher although PER was lower than that reported by Aly et al. (2017) using molasses and wheat bran as C sources. Furthermore, the FCR was similar while WG (%) was lower than those reported by Mansour and Esteban (2017), who used wheat milling and rice bran as carbon sources. The differences in WG (%) reported by Mansour and Esteban (2017) could be related to the size of the organisms and the culture period, since these authors cultured tilapia (*O. niloticus*) juveniles with an initial weight of 48.0 ± 1.10 g for a period of 10 weeks. Other authors have recently reported lower WG and FCR, and higher SGR and PER using sugar cane molasses as C source (Hisano et al., 2020); in this case, differences in SGR and PER could have been related to smaller size of fish used by Hisano et al. (2020). Kishawy et al. (2020) reported lower values in FCR, which could be explained in part by the greater volume of flocs, as well as higher protein and lipid content in the flocs reported in that work. Ferreira et al. (2015) have related greater volume of flocs with greater consistency in food availability, which can promote an increase in growth parameters. Other authors reported similar FCR, SGR, and factor K, and an increase in PER values in their treatments that showed greater water reuse in a biofloc system, attributing it to a greater presence of flocs available as food due to the low percentage of water change (Figueroa-Espinoza et al., 2022). In general, growth parameters indicate good fish performance in biofloc with fermented malted barley and molasses as C sources.

The treatment analysis in the present study did not show differences in any of the growth parameters between Bio-C, Bio-10%, and Bio-20% using fermented malted barley and molasses as C sources. The result shows

the possibility of partially replacing the diet to 20% with malted barley without negatively affecting fish performance.

The results in the present study agree with those of Estévez et al. (2021), who reported that the by-product of the spent grain brewery may be used at levels of 15%, without finding differences in growth, FCR, and fillet composition of sea bream (*Sparus aurata*) marine species. On the other hand, Durigon et al. (2020) reported higher final weight in the organisms fed with a higher level of protein in the diet; likewise, the organisms showed higher protein and lower lipid contents. The authors related a lower protein percentage and higher energy amount in the diet with higher lipid content and HI of the organisms. This result indicates that the intake of a diet with a lower protein:energy ratio could imply a greater deposition of energy reserves. Furthermore, other authors stated that higher HI in fish fed excess carbohydrates from by-products suggests increased lipogenic activity in the liver (Alves de Sousa et al., 2019), which has played an important role in energy reserve accumulation in lean fish (García-Moreno et al., 2021). In our case, the decrease in HI in the Bio-40% group could be related to a decrease in FI, which also tended to decrease with a higher percentage of diet replacement and could have reduced nutrient availability. Another factor that could affect available energy could be a low digestibility of the fermented malted barley. Hanley (1987) reported that the proteins and lipids of cereal by-products from the brewery have a significantly lower digestibility than those from fish meal. Although malted barley went through a fermentation process before its use, the digestibility was not verified in the present study. However, lower glucose values were observed in the groups with the highest diet replacement compared with the Bio-C group that did not contain malted barley as feed, which strongly supports this idea.

A higher percentage of diet substitution decreased growth parameters, which is comprehensible because of lower protein content in malted barley (17.84) regarding the control diet (38%). However, survival was not affected by the treatments. In fact, a cost–benefit analysis should be performed to elucidate to what extent a growth sacrifice can be compensated by a lower cost. Zerai et al. (2008) reported that in Nile tilapia juveniles (initial weight 25 g), replacing up to 50% of fishmeal with brewery waste did not show significant differences in growth. It should be noted that the fishmeal content of the control diet was 25% in the previous work (Zerai et al., 2008). Therefore, the amount replaced represented 12.5% of the diet, while in the present study, the replacement of up to 20% of the complete commercial diet with fermented malted barley did not show significant effects on the evaluated parameters. The biofloc chemical composition analysis showed higher protein content in Bio-C and Bio-10% regarding Bio-20 to Bio-40%. This result could have been reflected on higher protein content of diets due to no replacement and low replacement of the diet by fermented malted barley in Bio-C and Bio-10%, respectively, which may also have promoted greater growth of chemoautotrophic and heterotrophic communities. Lipid content was higher in the biofloc of Bio-C to Bio-20% regarding the Bio-30 and Bio-40% treatments; these data indicate higher nutritional contribution by the biofloc in Bio-C to Bio-20%, which could have contributed significantly to improving the biological parameters in these groups. According to the data in the present study, Brú-Cordero et al. (2017) reported a positive relationship between dietary and biofloc content protein, but Azim and Little (2008) reported no difference in nutritional quality of biofloc when different protein dietary contents were used. Brú-Cordero et al. (2017) suggested that biofloc chemical composition is more related to its biological composition than diet origin. However, in the present study, the microbiological communities present in the culture were not analyzed, so the effects observed in the Bio-30% and Bio-40% groups could be explained by the decrease in the two main macronutrients (proteins and lipids) in the biofloc, besides the fact that these two groups represent the highest percentage of diet replacement.

Our data showed similar protein content in muscle and whole fish in all the treatments, and the values were similar to those reported by Aly et al. (2017) using molasses and wheat bran as external C sources. On the other hand, lipids showed an increase only with B-30% in the muscle, while no differences were observed between treatments in the whole fish. Estévez et al. (2021) declared that partial replacement of fishmeal by brewery by-products (yeast and spent grain) produced significant changes in lipid but not in protein contents in *S. aurata* muscle. Lipids are the first component to be affected in fish when there are variations in dietary abundance and composition (Huss, 1988), which confirms that lipids are the favorite source of metabolic energy in fish (Sargent et al., 2002).

An analysis of pH, color, WHC, and profiles of AAs and fatty acids was carried out to determine the effect of the treatments on fillet quality. The pH results in this study were very similar to those reported by Pinto et al. (2019) in *O. niloticus* and Ntzimani et al. (2023) in European Sea bass (*Dicentrarchus labrax*), while Álvarez et al. (2020) reported slightly lower pH values in *S. aurata*. The pH of live fish muscle is close to 7.0. However, postmortem pH can vary from 6.0 to 7.1 depending on the season, species, and other factors (Simeonidou et al., 1998). The low range of variation in fish muscle pH after the sacrifice can be related to a low level of lactic acid production due to the low carbohydrate content in these organisms, which is in the order of 0.5% (Huss, 1988). The high pH levels observed could have been related to the high WHC observed in all the groups. At pH levels considered high (>6.0) or below the isoelectric point of actomyosin (approximately 5.0), the number of available charges increases, thus increasing the WHC (Gault, 1985). Likewise, a direct relationship was observed between the WHC values and the L* parameter values, where both were higher in Bio-C and Bio-40% and lower in groups with intermediate percentage of diet replacement by fermented malted barley.

The International Commission on Illumination (CIELAB) system allows a colorimetric characterization (throughout L*, a*, and b* color space) that has all the visible as well as non-visible colors and can be related to aspects of meat quality, such as health and animal welfare, which impacts the product value (Rodríguez-Tovar, 2017). Particularly, the component L* is related to the lightness of human vision; this value relates to the sample brightness, which can vary from 100 to 0, characterizing white and black, respectively (Konica, 1998). The observed values of L* showed similarity to those reported by Álvarez et al. (2020) in fillets of the *S. aurata*. However, they were lower than those shown by Pinto et al. (2019) in *O. niloticus* fillets and those reported by Ntzimani et al. (2023) in *D. labrax*. The a* coordinate defines the color tone and ranges from red (+a*) to green (−a*), and the b* coordinate represents the color tone from yellow (+b*) to blue (−b*) (Alves et al., 2008). The data in this study showed relatively high values of a* and b*, suggesting a reddish and yellowish hue of the fillets. Wang et al. (2017) reported that the muscle color and color stability of tilapia fillets became more yellowish during storage and suggested that this modification may be related to the yellow pigments that resulted from lipid and protein oxidation, contributing to the increase of b* values during the storage period. Our data in the present study partially support this theory, because two of the groups with the highest muscle lipid content (Bio-20 and Bio-30%) were the ones that showed higher b* levels along with Bio-10%. These results confirm that the b* value is an important indicator of the fish fillet quality and freshness (Mohan et al., 2016). Pinto et al. (2019) reported negative values of a* in fillets of *O. niloticus* indicating a green color, while the value of b* indicated a lighter yellow tone than that in our study. Likewise, Álvarez et al. (2020) reported values of a*, b*, c*, and H in *S. aurata* fed with partial substitution of dietary fish oil by vegetable oil, which were very different from those observed in tilapia. Data in this study provide important fillet quality characteristics of tilapia cultivated in biofloc; however, more studies are still needed to establish the standard characteristics of meat quality in fish. These parameters should be related to factors, such as diet composition or culture conditions. However, no clear trend was observed in the meat quality parameters in the present study (Table 6).

Partial dietary replacement increased the percentage of saturated fatty acids palmitic (C16:0) (Bio-30 and Bio-40%) and stearic (C18:0) (Bio-10 to Bio-40%) and decreased monounsaturated fatty acid oleic C18:1n9 (Bio-10%) in biofloc.

However, in the muscle, the high levels of dietary replacement decreased the percentage of ginkgolic saturated fatty acid (C13:0) (Bio-20 to Bio-40%), the palmitoleic monounsaturated fatty acid (C16:1n7) (Bio-20 and Bio-30%), and the arachidonic polyunsaturated acid (C20:4n6) (Bio-10 to Bio-40%) and eicosapentaenoic acid (C20:5n3).

The arachidonic and eicosapentaenoic fatty acids together with docosahexaenoic acid have been related to the production of immunologically active eicosanoids, affecting the ability to resist diseases (Lim & Webster, 2001). However, a major role of fatty acids in all the organisms is to generate metabolic energy in the form of adenosine triphosphate via mitochondrial β-oxidation. Lipids, and specifically fatty acids, are the favored sources of metabolic energy in fish for growth from the egg to the adult stage (Sargent et al., 2002). According to these authors, the fatty acids that are potential sources of metabolic energy in fish feeds include 16:0 and 18:1n-9; 20:1n-9 and 22:1n-11; and n-3 highly unsaturated fatty acids (HUFAs) 20:5n-3 and 22:6n-3. These fatty acids are heavily catabolized to

generate metabolic energy in fish, so they must be consumed in large amounts during growth of farmed fish species. Therefore, a greater effect on fatty acid reduction in the muscle in relation to the biofloc data can be explained by its use for the production of metabolic energy, especially in those groups with a higher percentage of dietary replacement. These data are supported by a significant correlation observed between the percentage of fatty acids C13:0, C21:0, C:18-1n9, C20:4n6, and C20:5n3 in muscle and the percentage of dietary replacement.

The AA methionine (Bio-30 and Bio-40%), serine (Bio-20 to Bio-40%), and taurine (Bio-40%) showed a decrease in muscle as the percentage of dietary replacement increased. Given that the methionine content was very similar between the commercial diet and the fermented malted barley, the decrease in these and other AAs in the muscle could be related to a lower intake rate of the fermented malted barley in the groups with a higher percentage of diet replacement.

However, no difference was observed in the muscle or in the whole fish protein content. Therefore, the decrease in some of the AAs could have been related to the use of free AAs for other physiological functions, such as the production of energy, given the decrease in Glu observed according to the increase in dietary replacement, whereas in the biofloc, a decrease was observed in the AAs His and Leu (Bio-20 to Bio-40%), Lys, Ser, and Gly (Bio-30 and Bio-40%). Since both fish excreta and uneaten food remains form the basis of nutrition for the establishment of bacterial communities in biofloc, the decrease in AAs in biofloc could have been related to a lower content of these acids in malted barley fermented compared to the commercial diet.

Met, His, Leu, and Lys constitute part of the essential AAs in the fish diet according to Halver and Hardy (2002). According to these authors, the percentage requirement of dietary protein for tilapia is 3.2% Met, 1.7% His, and 2.8–3.6% Leu, while the diet and the fermented malted barley contributed only to 0.17–0.18, 0.58–1.22, and 1.8–3.61% of Met, Hist, and Leu, respectively. However, the AA that was furthest from its requirement was Lys, for which according to Halver and Hardy (2002), it is 5.1% for tilapia and the fermented malted barley contributed only to 0.88% (Table 1). According to these authors, Lys appears to be the first limiting AA in feedstuffs commonly used in formulating feeds for warm water fish.

Reasonably, the decrease in growth, blood, and plasma parameters could have been related to an imbalance in the content of essential AAs, which showed a strong correlation with the percentage of dietary replacement. Nevertheless, the vast majority of these parameters were not affected by the Bio-20% diet, so up to 20% of the diet could be replaced by fermented malted barley without affecting the performance of the organisms, which could represent a very significant saving in fish production units, given that the cost of feed can represent more than 50% of the production costs (El-Sayed, 1999; FAO, 2009). Furthermore, supplementation of essential AAs in fermented malted barley should be explored in future studies to achieve an even higher percentage of dietary replacement.

In conclusion, the use of fermented malted barley and molasses as C sources allowed water quality parameters to be maintained in biofloc at values suitable for tilapia cultivation. No negative effects were observed on meat quality parameters of fish grown in biofloc with partial diet replacement. The biological parameters suggest that the diet can be partially replaced by fermented malted barley at a rate of 20%; however, blood and plasma parameters showed sensitivity from this level of diet replacement. Therefore, cost–benefit studies should be conducted to determine the optimal replacement percentage, as well as evaluate the long-term performance of organisms with partial dietary replacement by fermented malted barley.

ACKNOWLEDGMENTS

This research study was partially supported by the Universidad Autónoma de Baja California (UABC), México, through PRODEP (Programa para el Desarrollo Profesional Docente) for Professional Teacher Development and CONACYT (National Council for Science and Technology) México for Scholarship No. 790349 (Juan Ángel Gómez Cadena). The authors are also grateful to the company Maricultura del Pacífico for fish donation and the company Brew Capital Co. for malted barley donation; social service students and research assistants of the Aquatic Organisms Laboratory of the Research Institute in Veterinary Sciences (IICV) for their support during the bioassay;

Aquaculture Nutrition Laboratory of the Faculty of Marine Sciences (UABC) for their support in conducting chemical analyses; and D. Fischer for English edition.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding this research article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Miguel Cabanillas-Gámez https://orcid.org/0000-0001-8902-5084
Mario A. Galaviz https://orcid.org/0000-0002-6274-3024
Gustavo Alejandro Rodríguez-Montes de Oca https://orcid.org/0000-0002-5809-4586
Fernando Figueroa-Saavedra https://orcid.org/0000-0002-4893-7498
Lus M. López https://orcid.org/0000-0003-2371-7218
Idaly Trejo-Escamilla https://orcid.org/0000-0002-2499-7349
Fernando Barreto-Curiel https://orcid.org/0000-0001-5177-6632
Gilberto López-Valencia https://orcid.org/0000-0002-7557-5576

REFERENCES

Adineh, H., Naderi, M., Khademi, M., & Harsij, M. (2019). Biofloc technology improves growth, innate immune responses, oxidative status, and resistance to acute stress in common carp (*Cyprinus carpio*) under high stocking density. *Fish and Shellfish Immunology*, *95*, 440–448. https://doi.org/10.1016/j.fsi.2019.10.057

Aguirre-Guzman, G., Carvajal-de-la-Fuente, V., Neri-Coronado, M., Loredo-Osti, J., & Rábago-Castro, J. (2016). Cambios hematológicos y bioquímicos provocados por estrés agudo causado por manejo y captura de bagre (*Ictalurus punctatus*). *Revista MVZ Córdoba.*, *21*(2), 5345–5354.

Akinrotimi, O. A., Aba, O. M., Bckihclc, D. O., Udeme-naa, B., & Aranyo, A. A. (2010). Haematological characteristics of tilapia guineensis from Buguma creek, Niger delta, Nigeria. *EJEAFChe*, *9*(8), 1415–1422.

Álvarez, A., Fontanillas, R., Hernández-Contreras, A., & Hernández, M. D. (2020). Partial replacement of fish oil with vegetal oils in commercial diets: The effect on the quality of gilthead seabream (*Sparus aurata*). *Animal Feed Science and Technology*, *265*, 114504.

Alves, C. C. O., Resende, J. V., Cruvinel, R. S. R., & Prado, M. E. T. (2008). Estabilidade da microestrutura e do teor de carotenóides de pós obtidos da polpa de pequi (Caryocar brasiliense Camb.) liofilizada. *Ciência e Tecnologia de Alimentos*, *28*(4), 830–839. https://doi.org/10.1590/S0101-20612008000400011

Alves de Sousa, A., Pinho, S. M., Rombenso, A. N., Lemos de Mello, G., & Emerenciano, M. G. C. (2019). Pizzeria by-product: A complementary feed source for Nile tilapia (*Oreochromis niloticus*) raised in biofloc technology? *Aquaculture*, *501*, 359–367. https://doi.org/10.1016/j.aquaculture.2018.11.055

Aly, M. H., Zaki, M. A., Mansour, A. T., Srour, T. M., & Omar, E. A. (2017). Effect of Effective Microorganisms (Em) and Molasses, Wheat Bran and their Mixture in a Biofloc System on Microbial Protein Production, Water Quality, Growth Performance and Feed utilization of Nile Tilapia (*Oreochromis niloticus*) Fingerlings. *Journal of Animal and Poultry Production, Mansoura University*, *8*(11), 443–449. https://doi.org/10.21608/jappmu.2017.46059

Association of Official Analytical Chemists. (2000). *Official Methods of Analysis* (p. 1018). AOAC.

Atencio-Garcıa, V., Genes-López, F., Madariaga-Mendoza, D., & Pardo-Carrasco, S. (2007). Hematología y química sanguínea de juveniles de rubio (*Salminus affinis* PISCES: CHARACIDAE) del río Sinu. *Acta Biológica Colombiana*, *12*, 27–40.

Avnimelech, Y. (2009). Biofloc technology. In *A practical guide book* (1st ed., p. 272). The World Aquaculture Society. Baton Rouge. Society.

Avnimelech Y. (2011). Tilapia Production Using BioFloc Technology (BFT). Dept. of Civil & Environmental Eng.,Technion, Israel Inst. Of Technology Haifa, 32000, Israel. 4 pp.

Azim, M. E., & Little, C. L. (2008). The biofloc technology (BFT) in indoor tanks: wáter quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *283*, 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036

Bañuelos-Vargas, I., Rodríguez-Montes de Oca, G. A., Martínez-Montaño, E., Pèrez-Jimènez, A., Mendoza-Gamboa, O. A., Estrada-Godínez, J. A., & Hernández, C. (2021). Antioxidant and immune response of juvenile red tilapia (*Oreochromis sp*)

cultured at different densities in sea water with biofloc plus probiotics. *Aquaculture*, *544*, 737112. https://doi.org/10.1016/j.aquaculture.2021.737112

Barreto-Curiel, F., Focken, U., D'Abramo, L. R., Mata-Sotres, J., & Viana, M. T. (2019). Assessment of amino acid requirements for Totoaba macdonaldi at different levels of protein using stable isotopes and a non-digestible protein source as a filler. *Aquaculture*, *503*, 550–561. https://doi.org/10.1016/j.aquaculture.2019.01.038

Barreto-Curiel, F., Focken, U., D'Abramo, L. R., & Viana, M. T. (2017). Metabolism of *Seriola lalandi* during starvation as revealed by fatty acid analysis and compound-specific analysis of stable isotopes within amino acids. *PLoS One*, *12*(1), 1–17. https://doi.org/10.1371/journal.pone.0170124

Blaxhall, P. C., & Daisley, P. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, *5*, 771–781.

Boyd, C. E., D'Abramo, L. R., Glencross, B. D., Huyben, D. C., Juarez, L. M., Lockwood, G. S., McNevin, A. A., Tacon, A. G. J., Teletchea, F., Tomasso, J. R., Tucker, C. S., & Valenti, W. C. (2020). Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. *Jornal of the World Aquaculture Society*, *51*, 578–633. https://doi.org/10.1111/jwas.12714

Browdy, L. C., Ray, A. J., Leffker, J. W. & Avnimelech, Y. (2012). Biofloc-based aquaculture systems. En: Tidwell James, H. *Aquaqulture production Systems*. Wiley Blackwell. 278–307.

Brú-Cordero, S. B., Pertúz-Buelvas, V. M., Ayazo-Genes, J. E., Atencio-García, V. J., & Pardo-Carrasco, S. C. (2017). Biocultivo en biofloc de cachama blanca-*Piaractus brachypomus*- y tilapia nilótica-*Oreochromis niloticus*-alimentados con dietas de origen vegetal. *Revista de la Facultad de Medicina Veterinaria y Zootecnia*, *64*(1), 44–60. https://doi.org/10.15446/rfmvz.v64n1.65824

Buenaño, M. V. (2010). Hemograma de trucha arco iris (*Oncorhynchus mykiss*) en tres etapas de producción en la Cuenca alta de la provincia del Napo, Ecuador. *Bol Téc 9*, *Serie Zoológica*, *6*, 1–14.

Cequier-Sánchez, E., Rodríguez, C., Ravelo, A. G., & Zárate, R. (2008). Dichloromethane as a solvent for lipid extraction and assessment of lipid classes and fatty acids from samples of different natures. *Journal of Agricultural and Food Chemistry*, *56*, 4297–4303. doi:10.1021/jf073471e

Correa, A. D. S., Pinho, S. M., Molinari, D., Pereira, K. D. R., Gutierrez, S. M., Monroy-Dosta, M. D. C., & Coelho, M. G. (2020). Rearing of Nile tilapia (*Oreochromis niloticus*) juveniles in a biofloc system employing periods of feed deprivation. *Journal of Applied Aquaculture*, *32*, 139–156. doi:10.1080/10454438.2019.1679319

Crab, R., Defoirdt, T., Bossier, P., & Verstraete, W. (2012). Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture*, *356–357*, 351–356. https://doi.org/10.1016/j.aquaculture.2012.04.046

Crab, R., Kochva, M., Verstraete, W., & Avnimelech, Y. (2009). Bio-flocs technology application in over-wintering of tilapia. *Aquaculture Engineering*, *40*, 105–112. https://doi.org/10.1016/j.aquaeng.2008.12.004

De Macêdo, É. S., Urbich, A. V., Nakamura, J. S. T., da Cruz, T. P., Panaczevicz, P. A. P., Wernick, B., & Furuya, W. M. (2023). Effect of xylanase and β-glucanase on growth performance, activity of digestive enzymes, digestibility, and microbiome diversity of juvenile Nile tilapia fed soybean meal and/or sorghum distillers dried grains with solubles-based diets. *Aquaculture*, *565*(25), 739134. https://doi.org/10.1016/j.aquaculture.2022.739134

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*, *277*, 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019

Durigon, E. G., Lazzari, R., Uczay, J., Lopes, D. L. D. A., Jerônimo, G. T., Sgnaulin, T., & Emerenciano, M. G. C. (2020). Biofloc technology (BFT): Adjusting the levels of digestible protein and digestible energy in diets of Nile tilapia juveniles raised in brackish water. *Aquaculture and Fisherise*, *5*(1), 42–51. https://doi.org/10.1016/j.aaf.2019.07.001

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*, *257*, 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019

Ekasari, J., Angela, D., Waluyo, S. H., Bachtiar, T., Surawidjaja, E. H., Bossier, P., & De Schryver, P. (2014). The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture*, *426-427*, 105–111. https://doi.org/10.1016/j.aquaculture.2014.01.023

El-Sayed, A. F. M. (2019). *Tilapia culture* (2nd ed., p. 339). Academic Press.

El-Sayed, E. M. (2006). *Tilapia culture* (p. 294). CABI Publishing.

El-Sayed, M. A. F. (1999). Alternative dietary protein sources for farmed tilapia, *Oreochromis spp*. *Aquaculture*, *179*, 149–168. https://doi.org/10.1016/S0044-8486(99)00159-3

Emerenciano, M., Cuzon, G., Goguenheim, J., Gaxiola, G., & AQUACOP (2013). Floc contribution on spawning performance of blue shrimp Litopenaeus stylirostris. *Aquaculture Research*, *44*, 75–85. https://doi.org/10.1111/j.1365-2109.2011.03012.x

Estévez, A., Padrell, L., Iñarra, B., Orive, M., & San Martin, D. (2021). Brewery by-products (yeast and spent grain) as protein sources in gilthead seabream (*Sparus aurata*) feeds. *Aquaculture*, *543*, 736921. https://doi.org/10.1016/j.aquaculture.2021.736921

FAO, Organización de las Naciones Unidas para la Alimentación y la Agricultura. (2009). Cultured aquatic species fact sheets, *Oreochromis niloticus*. Retrieved from http://www.fao.org/tempref/FI/DOCUMENT/aquaculture/CulturedSpecies/file/es/es_niletilapia.htm

Fărcaş, A. C., Socaci, A. S., Mudura, E., Dulf, F. V., Vodnar, D. C., Tofană, M., & Salanţă, L. C. (2017). Exploitation of brewing industry wastes to produce functional ingredients. In M. Kanauchi (Ed.), *Brewing Technologies* (pp. 137–156). Intech Open.

Ferreira, L. M. H., Lara, G., Wasielesky, W., & Abreu, P. C. (2015). Biofilm versus biofloc: Are artificial substrates for biofilm production necessary in the BFT system? *Aquaculture International*, *24*, 921–930. https://doi.org/10.1007/s10499-015-9961-0

Figueroa-Espinoza, J., Rivas-Vega, M., Mariscal López, M., Emerenciano, M., Martínez-Porchas, M., & Baeza, A. M. (2022). Reusing water in a biofloc culture system favors the productive performance of the Nile tilapia (*Oreochromis niloticus*) without affecting the health status. *Aquaculture*, *558*(15), 1–9. https://doi.org/10.1016/j.aquaculture.2022.738363

Folch, J., Lees, M., & Stanley, G. (1957). A simple method of the isolation and purification of total lipids from animal tissue. *The Journal of Biological Chemistry*, *226*, 497–509.

García-Moreno, P. J., Jacobsen, C., Moltke, A.-D., & Yesiltas, B. (2021). *Omega-3 Delivery Systems Production, Physical Characterization and Oxidative Stability* (p. 491). Academic Press. https://doi.org/10.1016/C2019-0-03747-8

Gault, N. F. (1985). The relationship between water-holding capacity and cooked meat tenderness in some beef muscles as influenced by acidic conditions below the ultimate pH. *Meat Science*, *15*(1), 15–30. https://doi.org/10.1016/0309-1740(85)90071-3

Gujer, W., & Jenkins, D. (1974). A nitrification model for contact stabilization activated sludge process. *Water Research*, *9*(5), 5. https://doi.org/10.1016/0043-1354(75)90082-2

Hahn-Von-Hessberg, C. M., Grajales-Quintero, A., & Gutiérrez-Jaramillo, A. V. (2011). Parámetros hematológicos de Tilapia nilótica (*Oreochromis niloticus*, Linnaeus 1757) con peso entre 250 g y 350 g, en el Centro Experimental Piscícola de la Universidad de Caldas. *Revista Veterinaria y Zootecnia*, *5*(1), 47–61. Retrieved from https://revistasojs.ucaldas.edu.co/index.php/vetzootec/article/view/4500

Halver, J. E., & Hardy, R. W. (2002). *Fish Nutrition* (3rd ed., p. 839). Academic Press.

Hanley, F. (1987). The digestibility of foodstuffs and the effects of feeding selectivity on digestibility determinations in Tilapia, *Oreochromis niloticus* (L). *Aquaculture*, *66*, 163–179. doi:10.1016/0044-8486(87)90229-8

Haraz, Y. G., Shourbela, R. M., El-Hawarry, W. N., Mansour, A. M., & Elblehi, S. S. (2023). Performance of juvenile *Oreochromis niloticus* (Nile tilapia) raised in conventional and biofloc technology systems as influenced by probiotic water supplementation. *Aquaculture*, *566*, 739180. https://doi.org/10.1016/j.aquaculture.2022.739180

Hargreaves, J. A. (2013). *Biofloc Production Systems for Aquaculture*. Regional Aquaculture Center, SRAC Publication No. 4503 (pp. 1–12). SRAC Publ.

Hernández, M. L. E., Londoño, J. I. V., Hernández, K. A. G., & Torres, L. C. H. (2019). Los sistemas biofloc: una estrategia eficiente en la producción acuícola. *CES Medicina Veterinaria y Zootecnia*, *14*(1), 70–99. https://doi.org/10.21615/cesmvz.14.1.6

Hisano, H., Pinheiro, V. R., Losekann, M. E., & Guerra, M. S. (2020). Effect of feeding frequency on water quality, growth, and hematological parameters of Nile tilapia *Oreochromis niloticus* reared using biofloc technology. *Journal of Applied Aquaculture*, *33*(2), 96–110. https://doi.org/10.1080/10454438.2020.1715909

Hrubec, T. C., Cardinale, J. L., & Smith, S. A. (2000). Hematology and plasma chemistry reference intervals for cultured Tilapia (*Oreochromis Hybrid*). *Veterinary Clinical Pathology*, *29*(1), 7–12. https://doi.org/10.1111/j.1939-165x.2000.tb00389.x

Huss, H. H. (1988). *El pescado fresco: su calidad y cambios de calidad. Manual de capacitación preparado por cl Programa de Capacitación FAO/DANIDA en Tecnología Pesquera y Control de Calidad, No. 29* (p. 147). Colección FAO.

Khanjani, M. H., Alizadeh, M., Mohammadi, M., & Sarsangi Aliabad, H. (2021). Biofloc system applied to Nile tilapia (*Oreochromis niloticus*) farming using different carbon sources: Growth performance, carcass analysis, digestive and hepatic enzyme activity. *Iranian Journal of Fisheries Sciences*, *20*(2), 490–513. https://doi.org/10.22092/ijfs.2021.123873

Kishawy, A. T. Y., Sewid, A. H., Nada, H. S., Kamel, M. A., El-Mandrawy, S. A. M., Abdelhakim, T. A. M., El-Murr, A. E. I., Nahhas, N. E., Hozzein, W. N., & Ibrahim, D. (2020). Mannanoligosaccharides as a carbon source in biofloc boost dietary plant protein and water quality, growth, immunity and *Aeromonas hydrophila* resistance in Nile Tilapia (*Oreochromis niloticus*). *Animals*, *2020*(10), 1724. https://doi.org/10.3390/ani10101724

Kishida, N., Tsuneda, S., Sakakibara, Y., Kim, J. H., & Sudo, R. (2008). Real-time control strategy for simultaneous nitrogen and phosphorus removal using aerobic granular sludge. *Water Science and Technology*, *58*, 445–450. https://doi.org/10.2166/wst.2008.410

Konica, M. (1998). *Precise Color Communication - Color Control from Perception to Instrumentation*. JPN: Konica Minolta Sensing Inc.

Lim, C., & Webster, C. D. (2001). *Nutrition and Fish Health* (p. 393). Food Products Press.

Lucas, J. S., Southgate, P. C., & Tucker, C. S. (2019). *Aquaculture: Farming aquatic animals and plants* (p. 667). John Wiley & Sons Ltd.

Mansour, A. T., & Esteban, M. E. (2017). Effects of carbon sources and plant protein levels in a biofloc system on growth performance, and the immune and antioxidant status of Nile tilapia (*Oreochromis niloticus*). *Fish and Shellfish Immunology*, *64*, 202–209. https://doi.org/10.1016/j.fsi.2017.03.025

Marimuthu, V., Shanmugam, S., Sarawagi, A. D., Kumar, A., Kim, I. H., & Balasubramanian, B. (2022). A glimpse on influences of feed additives in aquaculture. *eFood*, *3*(1–2), e6. https://doi.org/10.1002/efd2.6

Martin, E. R., & Pagare, S. D. (2016). Partial replacement of fishmeal by animal protein with supplement of diets in freshwater fish Rasbora daniconius (Hamilton, 1822) and Puntius ticto (Hamilton, 1822). *Food and Feed Research*, *43*(1), 57–66.

Meraj, M., Yousuf, A. R., Bhat, F. A., Ali, M. N., Ganai, B. A., Shahi, N., Habiba, B., Ganaie, H. A., Shah, N. U. D., Rahman, M., & Rather, M. I. (2016). Hematological profiling of *Triplophysa marmorata* (Heckel 1838) from water bodies of Kashmir Himalaya—A perspective. *Journal of Fisheries and Aquatic Science*, *11*, 296–303. https://doi.org/10.3923/jfas.2016.296.303

Mohan, C. O., Ravishankar, C. N., & Srinivasagopal, T. K. (2016). Packaging interventions in low temperature preservation of fish—A review. *Food Processing & Technology*, *2*, 1–14. https://doi.org/10.15406/mojfpt.2016.02.00026

Monroy-Dosta, M. C., Lara-Andrade, R., Castro-Mejía, J., Castro-Mejía, G., & Cohelo-Emerenciano, G. (2013). Composición y abundancia de comunidades microbianas asociadas al biofloc en un cultivo de tilapia. *Revista de Biología Marina y Oceanografía*, *48*(3), 511–520. https://doi.org/10.4067/S0718-19572013000300009

Muñoz, V. N. (2018). *Contribución del biofloc inoculado con diferentes probióticos sobre el crecimiento y niveles de actividad enzimática digestiva en juveniles de tilapia (Oreochromis niloticus Var SPRING)* (p. 91). Centro de Investigación Científica y de Educación Superior de Ensenada, B.C.

Mussatto, S. I., Dragone, G., & Roberto, I. C. (2006). Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science*, *43*, 1–14.

NOM-062-ZOO, NOM (1999) Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio 58.

Ntzimani, A., Angelakopoulos, R., Semenoglou, I., Dermesonlouoglou, E., Tsironi, T., Moutou, K., & Taoukis, P. (2023). Slurry ice as an alternative cooling medium for fish harvesting and transportation: Study of the effect on seabass flesh quality and shelf life. *Aquaculture and Fisheries*, *8*, 385–392. doi:10.1016/j.aaf.2021.01.006

Oliveira, L. K., Wasielesky, W., & Tesser, M. B. (2022). Fish culture in biofloc technology (BFT): Insights on stocking density carbon sources, C/N ratio, fish nutrition and health. *Aquaculture and Fisheries*. In Press. https://doi.org/10.1016/j.aaf.2022.08.010

Oviedo, M., Brú, S., Atencio, V., & Pardo, S. (2013). Potencialidad de la región costera de Córdoba-Colombia-para el cultivo de tilapia nilótica. *Revista MVZ Córdoba*, *18*(3), 3781–3789.

Piazzon, M. C., Calduch-Giner, J. A., Fouz, B., Estensoro, I., Simó-Mirabet, P., Puyalto, M., Karalazos, V., Palenzuela, O., Sitjà-Bobadilla, A., & Pérez-Sánchez, J. (2017). Under control: how a dietary additive can restore the gut microbiome and proteomic profile and improve disease resilience in a marine teleostean fish fed vegetable diets. *Microbiome*, *5*(1), 1–23. https://doi.org/10.1186/s40168-017-0390-3

Pinto, L., Nascimento, K., Monteschio, J., Vital, A., Scapim, M., Madrona, G., & Prado, I. (2019). Effect of alginate-based edible coating with oatmeal on the quality of Nile Tilapia Fillets. *Chemical Engineering Transactions*, *75*, 589–594. https://doi.org/10.3303/CET1975099

Rey-Vásquez, G., & Guerrero, G. A. (2007). Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei: Perciformes). *Tissue & Cell*, *39*, 151–160. https://doi.org/10.1016/j.tice.-2007.02.004

Rodrigues, C. G., Garcia, B. F., Verdegem, M., Santos, M. R., Amorim, R. V., & Valenti, W. C. (2019). Integrated culture of Nile tilapia and Amazon River prawn in stagnant ponds, using nutrient-rich water and substrates. *Aquaculture*, *503*(30), 111–117. https://doi.org/10.1016/j.aquaculture.2018.12.073

Rodríguez-Tovar, J. M. (2017). *Caracterización de la Cichlasoma festae (Vieja Colorada) en la cuenca hidrográfica del Guayas* (p. 295). Ecuador.

Sáez, G., Chero, J., Cruces, C., Minaya, D., Rodriguez, C., Suyo, B., Romero, S., Guabloche, A., Tuesta, E., Alvariño, L., & Iannacone, J. (2018). Parámetros hematológicos y de bioquímica sanguínea en diez especies de peces marinos capturados por pesquería artesanal en la Bahía del Callao, Perú. *Revista de Investigaciones Veterinarias del Perú*, *29*(4), 1161–1177. https://doi.org/10.15381/rivep.v29i4.15204

Sánchez, I., Mejía, F., Huanuiri, K., Vásquez, J., Gonzales, A., & Fernández-Méndez, C. (2017). Respuesta hematológica y bioquímica en juveniles de paiche *Arapaima gigas* sometidos a diferentes concentraciones de amonio. *Folia Amazonica*, *26*, 51–58. https://doi.org/10.24841/fa.v26i1.418

Sargent, J. R., Tocher, D. R., & Bell, J. G. (2002). The Lipids. In J. E. Halver (Ed.), *Fish Nutrition* (2nd ed., p. 209). Academic Press.

Satheeshkumar, P., Ananthan, G., Senthil, K. D., & Jagadeesan, L. (2012). Haematology and biochemical parameters of different feeding behavior of teleost fishes from Vellar estuary, India. *Comparative Clinical Pathology*, *21*, 1187–1191. https://doi.org/10.1007/s00580-011-1259-7

Sgnaulin, T., Durigon, E. G., Pinho, S. M., Jer, M. J. G. T., de Lopes, D. L. A., & Emerenciano, M. G. C. (2020). Nutrition of Genetically Improved Farmed Tilapia (GIFT) in biofloc technology system: Optimization of digestible protein and digestible energy levels during nursery phase. *Aquaculture*, *521*, 734998. https://doi.org/10.1016/j.aquaculture.2020.734998

Sharma, B., & Ahlert, R. C. (1977). Nitrification and nitrogen removal. *Water Research*, *11*, 897–925.

Simeonidou, S., Govaris, A., & Vareltzis, K. (1998). Quality assessment of seven Mediterranean fish during storage on ice. *Food Research International*, *30*(7), 479–484. https://doi.org/10.1016/S0963-9969(98)00008-8

Sutton, D. S. (1997). *The influence of the Rendement Napole gene on pork quality*. *University of Illinois at Urbana-Champaign* (p. 9812785). ProQuest Dissertations Publishing.

Thomas, K. R., & Rahman, P. K. S. M. (2006). Brewery wastes. Strategies for sustainability. A review. *Aspects of applied biology*, *80*, 1–11.

Thrall, M., Weiser, G., Allison, R., & Cambell, T. (2015). *Veterinary Hematology and Clinical Chemistry*. *Wiley-Blackwell* (2nd ed., pp. 298–613). Iowa.

Velasco, P. N. (2019). *Evaluación de diferentes fuentes de carbono en sistema biofloc para la producción de juveniles de tilapia roja (Oreochromis spp.)* (p. 102). Universidad Nacional de Colombia.

Verdegem, M. C. J. (2013). Nutrient discharge from aquaculture operations in function of system design and production environment. *Reviews in Aquaculture*, *5*, 158–171. https://doi.org/10.1111/raq.12011

Wang, Z. C., Yan, Y., Nisar, T., Sun, L., Su, P., Chen, D.-W., & Guo, Y. (2017). Influence of postmortem treatment with nitric oxide on the muscle color and color stability of tilapia (*Oreochromis niloticus*) fillets. *Nitric Oxide*, *76*, 122–128. https://doi.org/10.1016/j.niox.2017.09.009

Wasielesky, W., Bezerra, A., Poersch, L., Hoffling, F. B., & Krummenauer, D. (2020). Effect of feeding frequency on the white shrimp *Litopenaeus vannamei* during the pilot-scale nursery phase of a superintensive culture in a biofloc system. *Journal of the World Aquaculture Society*, *51*(5), 1175–1191. https://doi.org/10.1111/jwas.12694

Yacout, D. M., Soliman, N. F., & Yacout, M. M. (2016). Comparative life cycle assessment (LCA) of Tilapia in two production systems: semi-intensive and intensive. *The International Journal of Life Cycle Assess*, *21*, 806–819. https://doi.org/10.1007/s11367-016-1061-5

Yilmaz, E. (2015). A review on hematology of fish and effect of dietary medicinal herb on blood parameters of fish summary. *Cumhuriyet Science Journal*, *36*(2), 37–50. https://doi.org/10.1111/jwas.12694

Zaretabar, A., Ouraji, H., Kenari, A. A., Yeganeh, S., Esmaeili, M., & Amirkolaee, A. K. (2021). One step toward aquaculture sustainability of a carnivorous species: Fish meal replacement with barley protein concentrate plus wheat gluten meal in Caspian brown trout (*Salmo trutta caspius*). *Aquaculture Reports*, *20*, 100714. https://doi.org/10.1016/j.aqrep.2021.100714

Zerai, D. B., Fitzsimmons, K. M., Collier, R. J., & Duff, G. C. (2008). Evaluation of brewer's waste as partial replacement of fish meal protein in Nile tilapia, *Oreochromis niloticus*, diets. *Journal of the World Aquaculture Society*, *39*(4), 556–564. https://doi.org/10.1111/j.1749-7345.2008.00186.x

How to cite this article: Cabanillas-Gámez, M., Gómez-Cadena, J., Galaviz, M. A., de Oca, G.-M., Figueroa-Saavedra, F., López, L. M., Trejo-Escamilla, I., Barreto-Curiel, F., & López-Valencia, G. (2024). Use of fermented malted barley by-product as partial feed replacement and carbon source for rearing Nile tilapia (*Oreochromis niloticus*) juveniles. *Journal of the World Aquaculture Society*, *55*(3), e13062. https://doi.org/10.1111/jwas.13062