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BIOFLOC TECHNOLOGY AND COCKROACH (*NAUPHOETA CINEREA*) INSECT MEAL-BASED DIET FOR NILE TILAPIA: ZOOTECHNICAL PERFORMANCE, PROXIMATE COMPOSITION AND BACTERIAL PROFILE

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Abstract

Different inclusion levels of cockroach meal *Nauphoeta cinerea* (CM) were investigated in diets for tilapia (*Oreochromis niloticus*) reared in biofloc systems in substitution of the soybean meal. Five treatments were evaluated (0, 5, 10, 15 and 20% of CM inclusion) using three experimental units per treatment. The experiment lasted for five weeks with units stocked with 10 juveniles (3.00±0.25 g) per replicate. Water quality, zootechnical performance, bacteriological profile, and proximate composition were analyzed and monitored. Zootechnical data was submitted to a regression analysis up to second order. No differences were verified regarding feed conversion, survival and productivity. The CM presented high protein levels (66.84%), high estimated gross energy (5270 kcal kg^{-1}), low lipids (6.07%) and mainly long-chain saturated fatty acids. Different bacteriological profiles were identified including species which may be potentially pathogenic and responsible for degrading organic matter. The overall results indicated that it is possible to include CM up to 10% in diets for tilapia juveniles raised in biofloc systems.

Key words: alternative ingredient, BFT, feed, microbial floc, protein

Aquaculture production has increased by 6.7% (average annual growth rate) in the last thirty years. In 2020, the total production has reached 87.5 million tons (FAO, 2022), in which tilapia and other cichlids increased from 379,169 t in 1990 to 6,100,719 t in 2020. Nile tilapia, *Oreochromis niloticus* itself represented 4.4 million tons, ranking third in global inland aquaculture (FAO, 2022).

Despite the rapid growth, there are few concerns regarding the aquaculture expansion. For instance, the reduction of effluent discharge, proper maintenance of water quality (farm and surrounding areas) and the exacerbated use of water and land are issues that need to be addressed (Wasielesky et al., 2006; Khanjani et al., 2023 a, b). For such purpose, the biofloc technology (BFT) system is considered a rational alternative in which high production yields can be achieved using less area and minimal or zero water renewal (Emerenciano et al., 2017). The maintenance of BFT is performed using continuous aeration and water movement enabling the formation of microbial aggregates that are mostly aerobic and heterotrophic (Ekasari et al., 2014). In addition, numerous dead and live microorganisms are present in bioflocs (e.g. nitrifying bacteria, protozoans, microalgae and invertebrates), along with feces, organic polymers and feed wastes (Khanjani et al., 2022 b, d). All combined, they serve as a food source to omnivorous aquatic species, such as tilapia (Emerenciano et al., 2013). Currently, the production of tilapia is successfully performed in BFT (Avnimelech, 2015; Khanjani et al., 2021, 2022 a) and the continuous availability of natural food source (bioflocs) may reduce the consumption of commercial diets by up to 30% (Avnimelech, 2007).

The evaluation of alternative ingredients for aquafeeds is essential for a sustainable long-term increase in fish production (Sousa et al., 2019). Conventional feedstuffs, such as soybean and fishmeal, are raw materials with unstable prices mostly due to demand in other sectors, such as poultry, swine, pets, and demand for the direct human consumption (FAO, 2009; Hardy, 2010; Cummins et al., 2017). Therefore, attention could be directed towards the locally available and cheaper protein source, and insect meal has been identified as one such ingredient (Lock et al., 2015; Katya et al., 2017). In this context, several studies have addressed new nutritional perspectives with alternative ingredients, such as insects (Sánchez-Muros et al., 2014; Freccia et al., 2020; Fabrikov et al., 2021). The commercial scale production of insect meals may provide some advantages such as (i) re-

liable nutritional quality; (ii) ability to be reared in small areas; (iii) less competition with other feed resources, and (iv) recycling of waste from diverse agri-food industries as a nutritional source for insect growth (Veldkamp et al., 2012; FAO, 2015; Khan et al., 2016; Ido et al., 2019). There are over 1 million known insect species worldwide; insects represent the largest and most diverse group within the Arthropoda phylum (Giribet and Edgecombe, 2019), but only a few species have been used for commercial purposes (Alfiko et al., 2022).

Some studies have been carried out using insects for aquafeeds or as a direct food item such as larvae of housefly (*Musca domestica*) for tilapia and African catfish (*Clarias gariepinus*) (Ogunji et al., 2008; Aniebo et al., 2009; Ezewudo et al., 2015); butterfly larvae (*Bematistes macaria*) to feed hybrid African catfish (*Heteroclarias*) (Nwamba and Ogunji, 2012); mealworm (*Tenebrio molitor*) for African catfish (Ng et al., 2001), gilthead seabream (*Sparus aurata*) (Piccolo et al., 2014), rainbow trout (*Oncorhynchus mykiss*) (Belforti et al., 2015), sea bass (*Dicentrarchus labrax*) (Gasco et al., 2016) and Nile tilapia (*O. niloticus*) (Sánchez-Muros et al., 2016); black soldier fly larvae (*Hermetia illucens*) for *S. aurata* (Oteri et al., 2022), Japanese eel (*Anguilla japonica*) (Kuo et al., 2022). Preliminary studies demonstrated that cockroach (*Nauphoeta cinerea*) meal can be used in tilapia feeds in clear-water recirculating aquaculture systems (RAS) (Freccia et al., 2016, 2020). In biofloc technology system, *Tenebrio molitor* insect meal has been evaluated for tilapia with positive outcomes (Tubin et al., 2020). In this sense, the present study evaluated different inclusion levels of speckled cockroach meal (CM) on zootechnical performance, proximate composition (CM and biofloc biomass) and bacteriological profile in tilapia juveniles reared in biofloc systems.

Material and methods

Local and biological material

The experiment was carried out at the Aquaculture Laboratory (LAQ), Santa Catarina State University (UDESC), Laguna, SC, Brazil. Tilapia juveniles (*O. niloticus*), weighing approximately 0.5 g, were obtained from a commercial hatchery and transported using specific bags to LAQ-UDESC. Once arrived, the animals were acclimatized over four weeks in three tanks (500 L of useful volume) using a biofloc technology (BFT) system (Emerenciano et al., 2012). Fish were fed with a commercial diet (32% of crude protein) up to the beginning of the trial. All procedures described below followed the Animal Ethics Committee Protocol (CEUA/UDESC, Protocol 1.45.14).

Experimental design and system

The treatments were characterized by five formulated diets containing increasing levels of speckled cockroach meal (CM): control (0%), 5, 10, 15 and 20% inclusion (Table 1), with three replicates per treatment totalling 15 experimental units. The CM was obtained from a private company (Nutrinsecta, Betim, MG, Brazil) specialized in producing several insect meals. The diets (calculated to be isoproteic, isoenergetic, isocalcitic and isophosphoric; Table 1) were formulated according to the species nutritional requirements proposed by Furuya (2010) and CM manufacturer information. The diets were manufactured at the Nutrition Laboratory of Aquatic Organisms (LANOA-UDESC). All ingredients were milled and sieved in a 300 µm mesh, weighed, blended and pelleted through a meat grinder. Pellets were dehydrated in an oven (55°C) for 36 hours and milled in order to obtain a particle size of approximately 2 mm.

In order to maintain the microbial community of the biofloc systems throughout the experimental period, subadults of tilapia *O. niloticus* (60–80 g individual weight and ~2 kg of total biomass) were cultivated using biofloc technology in one 1000 L polyethylene circular tank (defined as macrocosm). Four weeks prior to the beginning of the experiment, 300 L of inoculum (mature biofloc water) was obtained from a previous experiment and added into the macrocosm. The inoculum characteristics were TAN <0.5 mg L^{-1}, NO_2 <0.8 mg L^{-1} and settling solids ~10 mL/L. For the maintenance of the microbial biota (bioflocs) a protocol proposed by Emerenciano et al. (2012) was applied with a C: N ratio of 15:1 using sugarcane powder molasses and a commercial diet (22% CP) as the main sources of carbon and nitrogen respectively. This ratio was kept until the end of the experiment (35 days). The inputs from experimental diets were also considered.

The experimental units were stocked with ten *O. niloticus* juveniles (3.00±0.25 g) in a completely randomized experimental design with five treatments and three replicates. The experimental units (n=15) were composed of 26 L plastic tanks with a useful volume of 0.019 m^3 (defined as microcosms). The initial stock density was 10 fish/tank. The water used in the experiment (macrocosm and microcosms) was taken from the city's water supply network, dechlorinated (24 h with strong aeration) and kept at 28°C using 300-watt heaters (ratio of 1 W L^{-1}). In regards to water oxygenation, micro-perforated hose rings (70 cm length) were arranged centrally in the macrocosm's bottom. In each experimental unit, a porous stone (20 mm in diameter and 30 mm in height) was fixed to the middle of the tank. All aeration requirements were supplied by an air-blower (2 hp). Aiming to keep the same water quality and quali-quantitative profile of microorganisms in all units, the water inside the macrocosm was pumped (3500 L h^{-1}) to the experimental units (microcosms), and returned by gravity.

Water quality

Daily measurements (8:00 h) of pH (YSI-10A, Yellow Springs Instruments Inc., OH, USA), dissolved oxygen, temperature (YSI-55, Yellow Springs Instruments Inc., OH) and settleable solids (volume of bioflocs measured with an Imhoff cone) were performed in all

treatments. Once a week, the concentrations of ammonia (TAN), nitrite, nitrate and orthophosphate were measured with a photometer (ALFAKIT model AT 100P, Florianopolis – SC, Brazil). Total alkalinity was measured twice a week by volumetric titration using a commercial kit (ALFAKIT – 2058 and 2460, ALFAKIT, Florianopolis – SC, Brazil).

Zootechnical performance

Fish were fed three times a day (8:00, 13:30 and 18:00 h) and the diets were supplied according to the total biomass of fish (from 8% at the beginning of the experiment decreasing to 3% at the end) and adjusted weekly according to biometric results.

By the end of the experiment (5 weeks), the following zootechnical parameters were analyzed using formulas:

Final weight (FW) (g); weight gain: WG = (final weight – initial weight)

Feed conversion ratio (FCR) = feed consumption in a determined time period / weight gain

Specific growth rate (SGR) = (100 × {ln final weight – ln initial weight} / days of experiment

Survival (%) = (final number / initial number) × 100

Productivity (kg/m^{-3}) = (final biomass (kg) / water volume (m^3).

Bacterial community

Weekly samplings (15 mL Falcon tubes) were performed. The counting and characterization were performed according to methodology described by APHA (1992) and methodology adapted from Gutiérrez et al. (2016). From each sample, 1 mL was taken and diluted in sterile saline solution (1:10 dilution). One sub-sample of 0.1 mL was placed in agar plates MRS (Man Rogosa Sharpe), BHI (Brain Heart Infusion), TCBS (thiosulfate-citrate-bile salts), and TSA (trypticase soy), all in triplicates. The isolated strains were identified by the detection of the gene 16S (RNAr) by means of a genomic DNA extraction kit.

Table 1. Formulation and composition of the experimental diets with inclusion level of cockroach meal for tilapia reared in BFT system

Ingredients (%)	Inclusion level of cockroach meal (%)				
	0	5	10	15	20
Brazilian by-product fish meal	10.00	10.00	10.00	10.00	10.00
Cockroach meal	0.00	5.00	10.00	15.00	20.00
Soybean meal	47.10	40.10	33.20	26.30	19.50
Corn	21.28	23.48	25.48	27.58	29.80
Wheat bran	15.00	15.00	15.00	15.00	15.00
Fish oil	0.50	0.50	0.50	0.50	0.50
Soybean oil	2.10	2.00	2.00	1.90	1.70
Vitamin mineral premix	1.00	1.00	1.00	1.00	1.00
Vitamin C	0.10	0.10	0.10	0.10	0.10
Salt	0.50	0.50	0.50	0.50	0.50
Limestone	0.40	0.30	0.20	0.10	0.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Propionic acid[4]	0.02	0.02	0.02	0.02	0.02
Gelatin[5]	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Calculated composition[1]					
crude protein (%)	32.02	32.03	32.04	32.02	32.04
digestible energy (kcal kg^{-1})	3213	3221	3228	3239	3247
crude fibre (%)	4.28	4.33	4.39	4.45	4.5
calcium (%)	1.32	1.32	1.32	1.32	1.32
total phosphorus (%)	1.02	1.02	1.02	1.02	1.02
Analyzed composition[2]					
dry matter (%)	90.17	92.71	90.88	95.01	94.50
crude protein (%)	30.94	29.23	29.30	32.03	32.15
lipid (%)	12.08	10.82	12.19	12.93	13.98
ash (%)	8.81	11.01	11.43	9.54	9.26
gross energy (kcal kg^{-1})[3]	4883	4700	4755	4913	4982

[1]Values calculated for the chemical and energetic composition based on Furuya (2010).
[2]Values analyzed as the methodologies proposed by Silva and Queiroz (2002).
[3]Estimated value of gross energy. GE (kcal kg^{-1}) = [(crude protein × 5.65) + (lipid × 9.4) + (NNE × 4.15)] × 10
[4]Antioxidant; [5]Binder.

Proximate composition

At the end, proximate compositions (dry matter, ash, crude lipid, crude protein and energy) were performed on the CM, biofloc biomass and experimental diets (Table 1) according to methodologies proposed by Silva and Queiroz (2002). The gross energy was estimated by the equation GE (kcal kg^{-1}) = [(crude protein × 5.65) + (lipid × 9.4) + (non-nitrogen extract × 4.15)] × 10. For the biofloc sampling, the biomass was first concentrated by decantation and then dried in an oven at 55°C until reaching a constant weight. The analysis of the fatty acids profile was performed on the CM by gas chromatography according to AOAC (2000).

Statistical analysis

All data was analyzed for its variances normality and homogeneity and transformed by the equation x = arcsen when not normal (Zar, 1984). Results were evaluated according to the model: Yi = μ + Ti + ei in which: μ = general constant; Ti = cockroach meal level of inclusion, being i = 0%; 5%; 10%; 15%; 20%, and = random error. Once the effect was detected, the degree of freedom referred to the variables was unfolded in orthogonal polynomials in order to obtain the regression equations (Sokal and Rohlf, 1995). Regarding the variables that presented quadratic effects, the equations were derived for optimal point determination (Sokal and Rohlf, 1995).

Results

Water quality

The water quality values from the experimental units (microcosms) and the macrocosm were similar throughout the experimental period and averaged (mean values±standard deviation, maximum and minimum) as followed: dissolved oxygen (7.21±0.32, 7.72–6.26 mg L^{-1}); temperature (27.39±0.84, 28.80–25.20°C); pH (7.29±0.31, 7.87–6.8), settleable solids (20.89±8.18, 36.0–7.0 mL L^{-1}), TAN (1.42±0.70, 2.03–0.30 mg L^{-1}), nitrite (0.01±0.00, 0.01–0.00 mg L^{-1}), nitrate (0.67±0.21, 0.95–0.50 mg L^{-1}), orthophosphate (33.73±18.95, 50.0–13.1 mg L^{-1}) and alkalinity (23.40±2.97, 28.0–20.0 mg L^{-1}).

Zootechnical performance

The zootechnical performance (Table 3) indicated a quadratic effect ($P<0.05$) for final weight, weight gain (WG) and specific growth rate (SGR). Regarding WG and SGR, the results indicated 8.48% and 8.71% as the optimum inclusion levels (Figure 1). No differences were verified for the feed conversion ratio, survival and productivity ($P>0.05$).

Bacterial community

The results of bacterial sampling (Table 4) verified 10 genera/species of bacteria, in which three genera/species were considered as potentially pathogenic and seven genera/species as responsible for organic matter degradation. The pathogenic bacteria presented a low coefficient of variation (e.g., 11.4% for *Aeromonas hydrophila*) and an average abundance of 2.9% indicating low temporal variation in the system's bacterial community. Higher counts were detected for *Citrobacter freundii* with an average of 101.4×10^8 cells mL^{-1}. Regarding the degrading bacteria group, higher counts were detected for *Pseudomonas* sp. and *Enterobacteriaceae* with averages of 342 and 324×10^8 cells mL^{-1}, respectively. Some species (e.g., *Enterobacter amnigenus*) presented oscillations of up to 61.7% (CV), while the whole group averaged 7.6%. The percentage of pathogenic bacteria to degrading ones was lower than 20% during all experimental period with an average of 17.5%.

Table 2. Proximate composition (based on dry matter) and fatty acid profile (% of total fatty acids) of speckled cockroach meal and bioflocs

Proximate composition	Cockroach meal	Bioflocs
Dry matter (%)	93.36	24.46
Ash (%)	4.85	15.00
Crude protein (%)	66.84	9.02
Lipid (%)	6.07	0.40
Gross energy (kcal kg^{-1})	5270	3683
Fatty acid profile		
C12:0 Lauric acid (%)	0.14	NA
C14:0 Myristic acid (%)	1.10	NA
C16:0 Palmitic acid (%)	0.00	NA
C18:0 Stearic acid (%)	3.88	NA
C18:1 Oleic acid (%)	0.00	NA
C18:2 Linoleic acid (%)	0.00	NA
C18:3 Linolenic acid (%)	0.54	NA
C20:4 Arachidonic acid (%)	0.17	NA
C20:5 Eicosapentaenoic acid (%)	0.00	NA
C22:6 Docosahexaenoic acid (%)	0.24	NA

NA = not analyzed.

Table 3. Zootechnical performance of tilapia fed diets with inclusion level of cockroach meal (CM) in biofloc system during 5 weeks

	Inclusion level of cockroach meal (%)					Effect		
	0	5	10	15	20	CV %	L	Q
Initial weight (g)	2.99	2.96	3.03	2.99	3.00	3.26	0.78	0.83
Final weight (g)	11.18	12.64	12.41	11.89	10.40	9.54	0.28	0.03
Weight gain (g fish^{-1})	8.19	9.68	9.38	8.90	7.40	12.99	0.28	0.03
FCR	1.20	1.10	1.15	1.18	1.35	12.46	0.20	0.15
SGR (% day^{-1})	3.77	4.13	4.02	3.93	3.55	7.50	0.26	0.04
Survival (%)	93.33	66.67	83.33	90.00	93.33	39.32	0.86	0.49
Productivity (kg m^{-3})	5.49	3.64	5.40	5.66	5.00	25.20	0.66	0.75

CV = coefficient of variation; L = linear effect L<0,05; Q = quadratic effect Q<0,05. WG = 8.3011+0.3045CM-0.0176CM² (R^2: 0.96); SGR = 3.7994+0.0711CM-0.0042CM² (R^2: 0.94).

Table 4. Temporal variation of the bacterial community (×10^8 cells mL^{-1}) in water from tilapia cultured under BFT conditions during 5 weeks

Bacteria (×10^8 cells mL^{-1})		Weeks					Average	CV (%)
		2	2	3	4	5		
Pathogenic potential	*Aeromonas hydrophila*	90	73	89	72	74	79.6	11.4
	Aeromonas punctata	72	78	71	63	75	71.8	7.8
	Citrobacter freundii	97	94	102	116	98	101.4	8.5
	Total	259	245	262	251	247	252.8	2.9
Degrading potential	*Pseudomona fluorescens*	241	260	244	216	320	256.2	15.2
	Pseudomona aeruginosa	135	142	139	151	147	142.8	4.4
	Pseudomonas sp.	401	488	330	241	250	342.0	30.5
	Enterobacter sp.	78	83	86	92	90	85.8	6.5
	Enterobacteriaceae bacterium	197	280	310	407	426	324.0	29.1
	Enterobacter amnigenus	168	207	247	298	0	184.0	61.7
	Enterobacter aerogenes	100	106	113	108	102	105.8	4.8
	Total	1320	1566	1469	1513	1335	1440.6	7.6
	Pathogenic to degrading ratio (%)	19.6	15.6	17.8	16.6	18.5	17.5	

CV = coefficient of variation.

Proximate composition of biofloc

The biofloc biomass presented a high value of ash (15.0%), low crude protein (9.02%), low lipids content (0.4%) and estimated gross energy of 3683 kcal kg^{-1} (Table 2). Proximate composition of CM included ash (4.85%), protein (66.84%), lipids (6.07%) and estimated gross energy (5270 kcal kg^{-1}). The fatty acids profile analyzed in the CM indicated mainly long-chain saturated fatty acids with a predominance of stearic (C18:0) and myristic acids (C14:0). Linolenic, arachidonic acid (ARA) and docosahexaenoic acid (DHA) presented values of 0.54, 0.17 and 0.24%, respectively (Table 2).

Discussion

Water quality

The water physicochemical parameters observed were within the recommended and acceptable values for the species (El-Sayed, 2006), and most of them in agreement with the expected ranges normally observed in BFT systems (Emerenciano et al., 2017) and BFT+tilapia (Brol et al., 2017; Durigon et al., 2019, 2020; Sgnaulin et al., 2020). The relatively lower alkalinity is likely related to the high heterotrophic bacteria or high solids (bioflocs) concentrations. However, those levels did not impact the fish growth as results were comparable with other BFT+tilapia studies (Durigon et al., 2019, 2020; Sgnaulin et al., 2020).

Zootechnical performance

The present study suggests dietary CM inclusion of 10% as suitable for tilapia juveniles cultured in BFT. Freccia et al. (2016) did not find differences in the performance of tilapia juveniles (~2 g as initial weight) fed with up to 20% of CM inclusion in clear water recirculating system (RAS). However, comparing the growth performance of both studies, it was possible to observe an improved SGR (3.88% > 3.50%) and FCR (1.20 < 1.85) in our study with bioflocs. Such results indicate the natural food (bioflocs) may boost the growth and act as a complementary nutrient source in diets containing CM.

This fact may also be linked to the natural probiotic effect of the biofloc microorganisms (Emerenciano et al., 2013, Avnimelech, 2015; Khanjani et al., 2023 a), increasing the competition with pathogens and the feed digestibility due to the action of extracellular enzymes secreted by bacteria and ingested by the fish (Durigon et al., 2019). The average survival was 85.34%, which corroborates the data found by Freccia et al. (2016), and is considered acceptable for tilapia juveniles raised in BFT systems (Martins et al., 2017; Durigon et al., 2020). Similar FCR values were found by Brol et al. (2017) culturing ~3 g red tilapia and grey tilapia juveniles in BFT; but better than ~3.4 observed by Azim and Little (2008) culturing ~100 g tilapia juveniles also in BFT.

According to performance results a CM inclusion rate of up to 10% is possible. The derivation of quadratic equations indicated optimum inclusion levels of CM from 8.48 to 8.71% (Figure 1) increasing the weight gain by 15% and the specific growth rate by 8% when compared to the control diet. The quadratic effect indicated limitation on CM inclusion, different outcome than observed by Freccia et al. (2016) with 20% as highest CM inclusion level. However, although not statistically different, this high level resulted in the lowest survival (78%), possibly indicating a negative impact on fish metabolism. Other factors such as feed formulation, genetics and environmental conditions may also explain the differences between these two studies.

In our study, the decrease in performance with levels greater than 10% may be related to several factors such as deficiency in essential amino acid (Cummins et al., 2017) and high chitin levels present in the cockroach exoskeleton (Sánchez-Muros et al., 2014). Chitin is an acetylated amino-polysaccharide, similar to cellulose, but with a greater number of hydrogen bonds established with surrounding polymeric chains, which has been reported to be indigestible for several fish species (Rust, 2002). Such arrangements cause extra resistance (Barker et al., 1998) and may negatively impact the digestion.

The ability of fish to degrade and digest chitin by the chitinase depends upon the fish species (Smith et al., 1989) and available gut bacteria (Katya et al., 2017). In red tilapia and Nile tilapia, dietary fishmeal could be significantly replaced with shrimp meal, which is a rich source of chitin, without any adverse effects on the fish performance (El-Sayed, 1998; Mansour, 1998; Katya et al., 2017). Conversely, in the other species of tilapia, *O. niloticus* × *O. aureus*, the inclusion of chitin decreased growth (Shiau and Yu, 1999). Shiau and Yu (1999) observed that tilapia fed diets containing purified chitin and chitosan, had reduced weight gain and feed conversion rates at the low inclusion level of 2% chitin. Due to the lack of CM composition studies, a proper estimation of the chitin percentage present in the experimental diets used in this study was not possible. On the other hand, Finke (2007) considered that general insects have 11.6 to 137.2 mg kg^{-1} of body chitin. For example, with an average of 74.4 mg kg^{-1} of chitin from the cockroach meal, the diets with 10% and 20% of inclusion would represent 0.74% and 1.50% of chitin content, respectively, corroborating with Shiau and Yu (1999). In addition, different studies point out that chitin is one of the limiting factors for insect-based diets for fish (Ng et al., 2001; Sánchez-Muros et al., 2014). The authors indicated that reduced consumption and nutrient availability are the major issues reducing the zootechnical performance and nutrient utilization by the fish (Kroeckel et al., 2012). Other factors, such as amino acid imbalance or deficiency, presence of mycotoxins, fat oxidation and presence of unknown anti-nutritional factors, may also limit the CM inclusion levels and affect overall performance (Ng et al., 2001; Makkar et al., 2014).

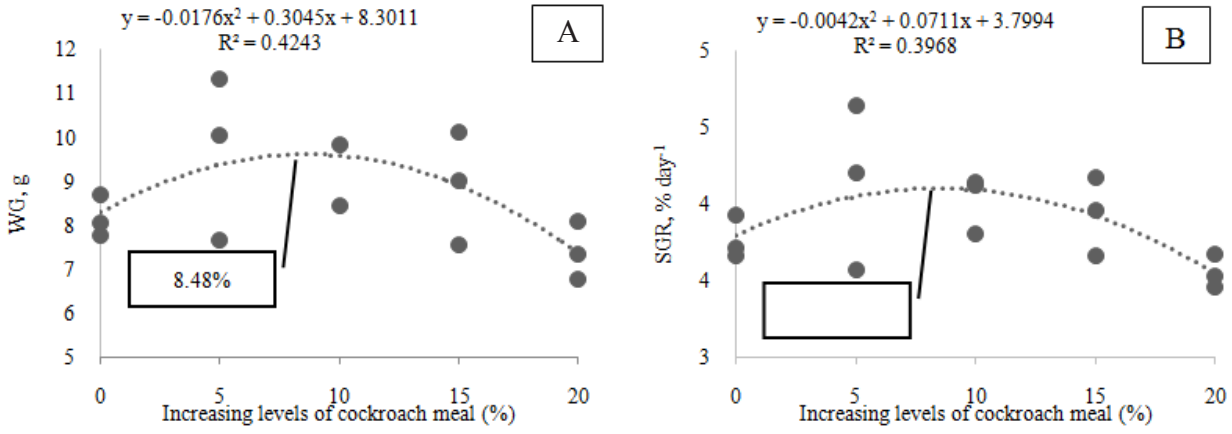


Figure 1. Weight gain (A) and specific growth rate (B) of Nile tilapia fed diets containing increasing levels of cockroach meal in biofloc system

Bacterial community

The percentage of pathogenic bacteria related to degrading ones was lower than 20% during the whole experimental period (~17.5% on average). In a practical sense this may mean the pathogenic bacteria were suppressed and the routine addition of a carbon source, that aimed to boost the heterotrophic bacteria community, may have controlled the pathogenic groups. Monroy-Dosta et al. (2013) using sugar cane molasses and rice by-products also controlled the pathogenic bacterial groups in a tilapia culture using BFT over 14 weeks. In the first 7 weeks, the authors observed a pathogenic-degrading ratio of <25% and after such period the ratio decreased up to 0%. The same trend was also observed in crustaceans BFT culture. Souza et al. (2014) demonstrated the addition of sugar cane molasses contributed to the maintenance of water quality and lower concentration of *Vibrio* spp. in *Farfantepenaeus brasiliensis* postlarvae cultured in BFT. In addition, Espírito Santo et al. (2017) evaluating an alternative carbon source (soybean molasses) demonstrated that soybean-to-sugarcane molasses ratios of 38–62% and 60–40% showed a significant *Vibrio* spp. reduction and could maintain water quality and zootechnical performance of *Litopenaeus vannamei* BFT culture. Crab et al. (2010) demonstrated the addition of live glycerol-grown bioflocs significantly increased the survival of brine shrimp *Artemia franciscana* larvae challenged to *V. harveyi.* The results above suggested that the bioflocs may have a biocontrol activity against the pathogenic bacteria. Besides the competition for nutrients/substrate, an additional benefit is related to the quorum sensing activity that might regulate virulence of vibrios towards different hosts (Crab et al., 2010). Certainly, complementary studies are needed to elucidate the bacterial dynamics in BFT.

Proximate composition

Cockroach meal (CM) seems to be a suitable crude protein source (66.84%) while limiting fatty acids sources (Table 2). Different protein levels have been reported in insect species as follows: silkworm pupae meal (60.7–81.7%), black soldier fly larvae (42.1–56.9%), housefly maggot meal (50.4–62.1%), yellow mealworm (52.8–82.6%), and lesser mealworm (57.3–62.6% dry matter) (Amza and Tamiru, 2017; Makkar et al., 2014; Mlček et al., 2018; Tran et al., 2015; Yu et al., 2018; Alfiko et al., 2022). However, in general, the protein content is similar or even higher than the main protein ingredients commonly used in aquafeeds, such as soybean meal, fishmeal, meat-and-bone meal, poultry by-product meal and others (Furuya, 2010; Oonincx and Finke, 2021; Alfiko et al., 2022).

On the other hand, insect meals may present lower lipid content as compared to fish meal (Alfiko et al., 2022). Insects accumulate lipid in their body, especially in their embryonic stages (Sánchez-Muros et al., 2014). However, the lipid levels and fatty acid profile in insect meals may change according to e.g. (i) stage of development and (ii) their food source (Makkar et al., 2014; Barroso et al., 2019), in which this last one can be manipulated aiming to boost certain fatty acids (Barroso et al., 2019).

In the present study, there was a dominant presence of saturated fatty acids although some n-3 (linolenic and DHA) and n-6 (ARA) fatty acids were also found. Such fatty acids are extremely important for both finfish and crustacean nutritional metabolism (Glencross, 2009). The values of DHA have been reported in insect species, banded cricket (0.07%), Jamaican field cricket (0.15%) black soldier fly larvae (0.03–1.66% of total lipids) (Alfiko et al., 2022). In our study, the level of ash for CM was 4.85%. Shin and Lee (2021) reported ash values for beetle meal (3.61%), white-spotted flower chafer (4.17%), mealworm (4.27%), two-spotted cricket (4.99%) and rice grasshopper (7.76%, based on dry matter). Normally, insects contain relatively low levels of ash due to the nature of their skeleton (Oonincx and Finke, 2021).

The proximate composition of the biofloc biomass revealed low levels of crude protein (9%) and lipid (0.4%). Long et al. (2015) detected values of 41.1% for crude protein, 1% for lipid, and 6.1% for ash. Khanjani et al., (2022 c) observed values of 26.38 to 28.97% for protein, 0.84 to 1.02% for lipid and 31.53–36.42% for ash. Compared to other studies, our results were relatively lower (for further review, see Martínez-Córdova et al., 2016) and may have impacted the ability of CM inclusion (e.g. >10%). In a similar approach but testing the inclusion of pizzeria by-product, Sousa et al. (2019) were able to include up to 20% of pizzeria by-product meal in diets for Nile tilapia reared in BFT without performance losses. The biofloc composition may vary according to the carbon source used, light intensity, microbial profile (phytoplankton to bacteria ratio), salinity, carbon to nitrogen ratio, species/feed source and others (Martínez-Córdova et al., 2015; Khanjani and Sharifinia, 2022 a, b; Khanjani et al., 2022 b). As tilapia continuously ingest the bioflocs (Avnimelech, 2007; Azim and Little, 2008; Ekasari et al., 2014; Mabroke et al., 2021), the continuous availability of a natural food source (bioflocs), its microbial composition of bioflocs and nutritional profile may have direct impact on the dietary inclusion levels of alternative ingredients in BFT rearing conditions.

Conclusion

The results indicated that it is possible to include CM up to 10% in diets for tilapia juveniles raised in biofloc systems without losses in zootechnical performance; and CM could be a promising alternative replacing key ingredients, e.g. soybean meal in tilapia feeds. The regression analysis showed 8.6% as the optimum inclusion level considering the weight gain and specific growth rate in our experimental conditions. Considering the need of more sustainable approaches in the aquaculture sector, more studies focused on insect meals evaluating different species/phases/culture systems are strongly encouraged.

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Availability of data and material

The authors certify that all data used to reach the findings are available within the article results. Data available on request from the authors.

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