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Effects of dietary protein content on hybrid tilapia (*Oreochromis aureus* × *O. niloticus*) performance, common microbial off-flavor compounds, and water quality dynamics in an outdoor biofloc technology production system

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ABSTRACT

Given tilapia grown in the biofloc technology production system can consume the biofloc, it should be possible to optimize formulated diet protein content to account for nutrition derived from consuming biofloc. The present study, conducted in an outdoor biofloc technology production system, evaluated impacts on fish production indices, common microbial off-flavors, and water quality dynamics for hybrid tilapia (*Oreochromis aureus* × *O. niloticus*) fed diets formulated to contain 22.5%, 27.7%, and 32.3% digestible protein (DP) and 6% lipid. Fingerlings (32.2 ± 10.1 g/fish) were stocked in tanks (18.6 m^2; 16.6 m^3) in May 2016 at 25/m^2 (29/m^3) and grown for 5 months to market size. At harvest, fish fed the 22.5% DP diet were significantly smaller (518 g/fish) and had significantly higher feed conversion (1.5) than those fed the higher DP diets (553–564 g/fish and 1.4, respectively). Feed nitrogen input and nitrification rate increased linearly with increased DP. Results of this study suggest that by using ideal protein theory to formulate diets supplemented with the first four limiting amino acids (Lys, Met, Thr, Ile) digestible protein can be reduced from 32.3% to 27.7% without adversely affecting hybrid tilapia productivity indices. Market size distributions, nutrient retention, 2-methylisoborneol and geosmin off-flavors, and pond water quality dynamics in relation to diet DP also are discussed.

1. Introduction

The biofloc technology (BFT) production system is used to intensify aquaculture production because water quality conditions conducive to rapid growth are maintained for the densely stocked and intensively fed culture species. In the heterotrophic-based BFT system exogenous organic carbon, an energy and carbon source, is added to stimulate bacterial assimilation of excreted feed nitrogen (total ammonia-nitrogen, TAN) (Avnimelech et al., 1989; Avnimelech et al., 1992; Avnimelech, 1999). Tilapia can consume this microbial biomass, which can substitute for protein in the formulated ration, thereby increasing overall efficiency of protein utilization (Avnimelech et al., 1989; Avnimelech et al., 1992; Avnimelech, 2007; Avnimelech and Kochba, 2009). Much subsequent published research on the tilapia BFT production system focuses on the efficacy of various types of organic matter (e.g., Caipang et al., 2015; Li et al., 2018) and/or the optimal carbon:nitrogen (C:N) ratio (Perez-Fuentes et al., 2016; Liu et al., 2018b). Surprisingly, only two published studies (Azim and Little, 2008; da Silva et al., 2018) were found that evaluated formulated diet protein levels for growing fingerling- or juvenile/stocker-size tilapia in the BFT production system. In both studies, exogenous organic carbon was added daily (Azim and Little, 2008) or periodically (da Silva et al., 2018). No published reports were found that evaluated protein content of formulated diets for BFT production of market-size tilapia.

A combined photoautotrophic-, chemoautotrophic-based BFT production system, in contrast to the heterotrophic-based BFT system, does not rely on repeated, frequent application of organic carbon to control TAN. Rather, phytoplankton assimilation mainly controls TAN initially and after about 30 d TAN is controlled primarily by nitrification and secondarily by phytoplankton assimilation (Green et al., 2014).

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However, up to several applications of exogenous organic carbon may be necessary to mitigate the spike in TAN concentration that occurs at the onset of nitrification. Continued application of exogenous organic carbon to the BFT system will favor heterotrophic bacteria because of the high C:N ratio, whereas nitrifying bacteria are favored at low C:N ratios (Luo et al., 2017; Liu et al., 2018b). Suspended solids (a complex of living organisms closely associated with particulate organic matter) accumulate in culture water in response to high feeding rates in both BFT systems and from repeated carbon additions in the heterotrophic BFT system. A side-stream settling chamber often is used to manage total suspended solids (TSS) concentration.

Ideal protein (IP) theory has been used to formulate diets for production of fingerling or juvenile tilapia in aquaria, recirculating aquaculture system (RAS) tanks, or net pens (Furuya et al., 2004, 2005; Botaro et al., 2007; Bomfim et al., 2008). For example, Furuya et al. (2005) fed diets formulated at 25.5%, 27%, 28.5% and 30% digestible protein (DP) to juvenile Nile tilapia and were able to reduce dietary protein to 27.5% in fish grown from 5 g to 125 g based on quadratic regression of responses. In that study, each of the three reduced DP diets was supplemented with Met, Lys, and Thr (first-three limiting amino acids) at the same level as that of the 30% DP control diet, which was formulated on an ideal protein basis using the whole-body amino acid profile. Similarly, Botaro et al. (2007) fed diets formulated at 27%, 25.2%, 24.3% and 22.7% DP to juvenile tilapia (35 g initial weight) reared in net pens to about 250 g and were able to reduce DP to 24–25%, depending on response variable, by supplementing the first three limiting amino acids at the same level as that of the 27% DP control diet.

An alternative philosophy for reducing intact protein in the diet is to use a balanced amino acid ratio while reducing DP versus supplementing the limiting AA to target an absolute quantity of AA based on an estimated protein requirement. Therefore, one can increase the number of supplemental limiting amino acids included in the formula targeting the IP concentrations for those amino acids at each level of reduced DP, rather than at the highest (or control) level of the series (Rawles et al., 2012). Thus, both intact protein and the level of supplemental amino acids included in the diet are reduced, potentially decreasing diet cost and excess nutrient excretion (Chowdhury et al., 2013). Since ideal protein theory has not been used to formulate diets for fast-growing tilapia to market size in a BFT production system, we tested the hypothesis that significant reduction in protein could be achieved in hybrid tilapia (*Oreochromis aureus* × *O. niloticus*) reared to market size (454 g/fish) in an outdoor, photoautotrophic/chemoautotrophic BFT production system without constant organic carbon additions by supplementing the first four limiting amino acids (Lys, Met, Thr, Ile) and formulating to the IP profile (muscle) at each level of tested DP.

2. Materials and methods

2.1. Experimental design and diet

Using a completely randomized design in triplicate outdoor tanks, hybrid tilapia were fed three practical test diets for 5 months (Table 1). Diets were formulated at USDA-ARS-HKDSNARC (Stuttgart, AR USA) according to Gaylord and Rawles (2005) to contain one of three digestible protein (DP) levels (22.5%, 27.7%, 32.3%). Diets were supplemented on an IP basis with the first four limiting amino acids (Lys, Met, Thr, Ile) according to the tilapia muscle amino acid profile at each of the targeted diet DP levels (Table 2). The muscle amino acid profile used was an average of tilapia muscle samples analyzed in our lab and those of Furuya et al. (2010). Composite averages of nutrient composition and apparent digestibility coefficients (ADCs) of nutrients in each of the dietary ingredients were obtained from the literature and analyses in our labs and used for diet formulation (see Appendix). As dietary DP increased, the percent contribution of each of the seven main protein sources to the total DP contributed by those ingredients was held as constant as possible to limit variability in ingredient effects. Corn and wheat levels were allowed to vary in an inverse fashion to obtain final balance of DP and digestible energy (DE) among test diets. As dietary DP increased, corn inclusion decreased from 33.9% to 9.4% and contributed 2.7% to 0.75% DP. Wheat inclusion increased slightly from 24% to 26% and contributed 2.5% to 2.75% DP. DE was targeted at 13.39 MJ/Kg diet; lipid was targeted at 6% (dry weight basis) from feedstuffs and supplemental soybean oil (1.7–1.1% of diet), however, actual analyzed lipid levels were slightly lower at 4.8–4.9% (dry weight basis, dwb). Available phosphorus was targeted at 0.6% from feedstuffs and supplemental dicalcium phosphate for total formulated P levels of 0.88 to 0.92% (dwb), while total P levels measured in the diets were 0.90 to 0.98% (dwb). Except for amino acids, ingredients were sourced by Rangen, Inc. (Buhl, ID USA). Amino acids were provided by Evonik Industries (Essen, Germany). Test diets were manufactured by Rangen, Inc. with commercial methods using a twin-screw cooking extruder to produce 4.0-mm floating pellets. Upon receipt at HKDSNARC, bagged diets were stored in a temperature controlled (18–20 °C) feed room until used.

Table 1
Formulations and composition (g/kg as-is) of commercially extruded practical test diets fed to hybrid tilapia in a biofloc production system.

	Intact digestible protein (%)		
Ingredient[a]	22.5	27.7	32.3
Menhaden fish meal – Select™	17.2	23.5	29.4
Poultry by-product meal – pet-food grade	33.8	46.1	57.7
Soybean meal 48%	59.0	80.5	100.7
Distillers Dried Grains (DDG)	113.2	154.5	193.3
Soy protein concentrate	44.4	60.7	75.9
Blood meal, spray dried poultry	36.8	50.3	62.9
Feather meal, hydrolyzed	20.8	28.4	35.5
Corn	349.0	217.7	97.3
Wheat, soft white	244.0	256.4	267.0
Soybean oil	15.2	12.4	9.7
Stay-C 35™	1.4	1.4	1.4
Vitamin premix[b]	9.1	9.1	9.1
Mineral mix[b]	0.9	0.9	0.9
Sodium chloride	2.5	2.5	2.5
Magnesium oxide	0.5	0.5	0.5
Potassium chloride	5.1	5.1	5.1
Dicalcium phosphate	23.5	22.7	21.5
Choline chloride 60%	7.6	7.6	7.6
DL-methionine[c]	1.9	2.5	3.0
Lysine HCl[c]	9.8	12.0	13.4
Threonine[c]	1.4	1.8	1.9
Isoleucine[c]	1.4	1.9	2.2
Wheat, mill run	0.5	0.5	0.5
Mold inhibitor	1.0	1.0	1.0
Analyzed Composition (as-is)			
Crude protein (N × 6.25), g/kg	266.1	313.0	359.9
Crude fat, g/kg	44.0	43.9	44.8
Gross energy, MJ/kg	19.51	19.71	19.93
Phosphorus, g/kg	8.06	8.38	9.03
Moisture, g/kg	103.3	83.7	83.5
Ash, g/kg	64.9	68.5	75.1

[a] Except where noted all ingredients sourced by Rangen, Inc. (Buhl, ID USA).
[b] Rawles et al. (2018).
[c] Amino acids provided by Evonik Industries, Essen, Germany.

2.2. Tank management, fish, and feeding

The feeding trial was conducted outdoors in nine wood-framed rectangular BFT production tanks (18.6 m^2, 16.6 m^3) with a slightly sloped bottom and lined with high-density polyethylene (Fig. 1). Two 6.3-kW high-efficiency regenerative blowers (Sweetwater model SST70, Pentair Aquatic Ecosystems, Apopka, FL USA) in parallel supplied air continuously to two diffuser grids (high-efficiency antimicrobial diffuser tubing, Pentair Aquatic Ecosystems, Apopka, FL USA) on the bottom of each tank. Two conical-bottom settling chambers (each 130 L, 117 L operating volume) connected in series were installed at each tank. Water

Table 2
Analyzed amino acid (AA) composition (g/kg, as-fed) of tilapia muscle (ideal protein, IP) and the test diets, percent difference from IP (*in italics*), and sum of squared amino acid deviations (SS Dev) in the diet compared to the IP.

DP[a]	22.5		27.7		32.3	
	Muscle	Diet	Muscle	Diet	Muscle	Diet
Ala	13.44	17.25 *(28.4)*	16.54	19.95 *(20.6)*	19.29	21.65 *(12.2)*
Arg	12.47	15.36 *(23.1)*	15.36	18.55 *(20.8)*	17.91	20.73 *(15.8)*
Asx	22.40	22.02 *(−1.7)*	27.58	25.80 *(−6.4)*	32.16	31.86 *(−0.9)*
Glu	33.98	47.17 *(38.8)*	41.83	54.70 *(30.8)*	48.77	62.51 *(28.2)*
Gly	12.03	12.31 *(2.3)*	14.81	14.80 *(−0.1)*	17.27	17.07 *(−1.2)*
His	5.74	8.32 *(44.9)*	7.07	10.01 *(41.6)*	8.24	11.42 *(38.5)*
Ile	9.60	11.10 *(15.6)*	11.82	13.12 *(11.0)*	13.78	15.38 *(11.6)*
Leu	17.48	24.23 *(38.7)*	21.51	27.60 *(28.3)*	25.09	32.57 *(29.8)*
Lys	19.34	21.67 *(12.1)*	23.80	26.96 *(13.3)*	27.76	27.10 *(−2.4)*
Met	5.67	3.90 *(−31.1)*	6.98	5.00 *(−28.4)*	8.14	5.63 *(−30.8)*
Phe	9.36	13.84 *(47.9)*	11.52	16.26 *(41.1)*	13.43	20.96 *(56.1)*
Ser	8.00	12.25 *(53.1)*	9.85	14.82 *(50.4)*	11.49	17.39 *(51.3)*
Thr	10.29	11.90 *(15.6)*	12.67	13.70 *(8.2)*	14.77	17.18 *(16.3)*
Tyr	6.44	7.66 *(18.9)*	7.93	8.99 *(13.3)*	9.25	11.03 *(19.2)*
Val	11.16	14.13 *(26.6)*	13.74	16.66 *(21.2)*	16.02	19.26 *(20.2)*
SS Dev (all AA)	*311.42*		*309.82*		*388.77*	
SS Dev (unsuppl. AA)[b]	*298.03*		*293.14*		*373.68*	

[a] Diet designations are % digestible protein (DP).
[b] SS Dev of the unsupplemented AA (without Met, Lys, Thr, and Ile).

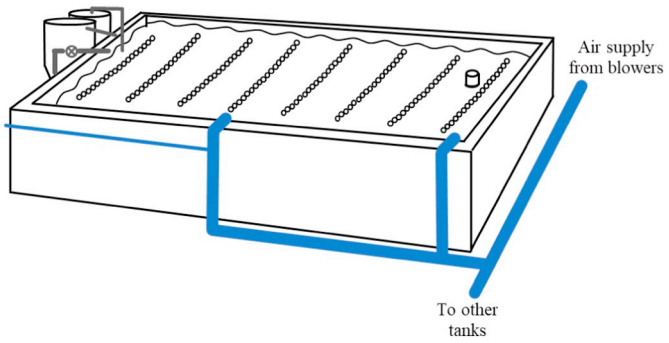

Fig. 1. Wood-framed, high-density polyethylene-lined biofloc technology production system tank (6.10 m × 3.05 m inside dimensions). Air from blowers supplies two aeration grids located on the bottom of each tank. Side stream air supplies air lift pump that moves water from tank to the first settling chamber (117 L), then flows by gravity to the second settling chamber (117 L) and back to the tank. Not to scale.

moved to the first settling chamber by airlift (ca. 3 L/min) and flowed by gravity through the second chamber to the tank (hydraulic retention time = 78 min). Settling chambers were operated continuously beginning day 22 (9 June) and flow rate was quantified at least once daily; individual chambers were drained completely as needed.

On 4 May 2016, approximately 2.5 m^3 of water from a HKDSNARC pond with a phytoplankton bloom were added to each tank, and then filled with ground water (226 mg/L as $CaCO_3$ total alkalinity). Pond water was used to expedite development of a phytoplankton bloom to aid in removal of TAN. Each tank received a total of 0.35 kg urea fertilizer (46-0-0, N-P-K) from 5 to 11 May, followed by addition of 6.8 kg calcium chloride and 4.5 kg sodium chloride (to raise chloride concentration to at least 600 mg/L) on 11 May, and a total of 5.1 kg dried molasses (Sweet45, Westway Feed Products, New Orleans, LA USA) from 1 to 2 June. Dried molasses was the only source of exogenous organic carbon added to tanks to stimulate bacterial transformation of TAN (Avnimelech et al., 1992; Avnimelech, 1999). Sodium bicarbonate (1.13 kg/tank) was added as needed to maintain pH above pH 7.0. Tanks were not flushed, but water was added to tanks as needed to replace losses from evaporation and draining of the settling chamber.

All-male hybrid tilapia fry (ca. 0.8 g) were sourced from Aquasafra, Inc. (Bradenton, FL USA) in March 2016 and reared indoors until stock out. Fish were graded through a No. 38 bar grader, held overnight, and stocked into BFT tanks at 25 fish/m^2 (29 fish/m^3) on 18 May 2016. All fish were counted by hand to ensure accurate stocking data. Initial weight was 32.2 ± 10.1 g/fish (mean ± SD) and initial biomass was 0.9 ± 0.01 kg/m^3.

Fish were fed daily to apparent satiation a 40% crude protein/14% fat commercial extruded diet (EXTR400, Rangen, Inc., Buhl, ID USA) from stocking through 9 June 2016 (22 days) when the test diets arrived. Mean fish weight and estimated fish biomass did not differ significantly among treatments and averaged 73.5 ± 2.0 g/fish ($P = 0.519$) and 2.1 ± 0.1 kg/m^3 ($P = 0.770$), respectively, when feeding of test diets began (10 June 2016). Fish were fed their randomly assigned test diet by hand to apparent satiation twice daily during the week and once daily on weekends from 10 June to 9 October 2016 (122 days) and the quantity recorded. Feed conversion ratio (FCR) was calculated for each tank as the total quantity of feed fed (dry matter basis) divided by the net (wet) weight of fish harvested. The experimental protocol was approved by the HKDSNARC Institutional Animal Care and Use Committee (Approval Number 2016–001.1) and conformed to ARS Policies and Procedures 130.4 and 635.1.

Dissolved oxygen and water temperature in each tank were monitored continuously as described by Green et al. (2014).

Water samples from each tank and from each settling chamber's effluent were collected between 0700 and 0800 h beginning 19 May 2016 and 15 June 2016, respectively, and continuing weekly. Samples were analyzed as described by Rawles et al. (2018) for total ammonia-nitrogen, nitrite-nitrogen (nitrite-N), nitrate-nitrogen (nitrate-N), soluble reactive phosphorus (SRP), total alkalinity, settleable solids, total suspended solids (TSS), settleable solids (SS), chlorophyll *a*, and pH.

The effect of the settling chambers on changes in water quality variable concentrations was calculated weekly as the difference between settling chamber influent and effluent concentrations multiplied by the total volume of water that flowed through the chambers each week. Settling chamber influent concentration was assumed to be the same as tank water concentration. Net changes in water quality variable concentrations were adjusted for losses to draining of settling chambers, which were calculated as the volume drained multiplied by tank or effluent water quality variable concentration for the primary or secondary settling chamber, respectively.

2.3. Fish and tissue sampling

Twenty-two fish were selected at random from the initial population at stocking, euthanized, and frozen for later analysis of whole body composition. Samples of at least 150 fish/tank to estimate growth trajectories, taken on days 23, 51, 79, and 106, were weighed in bulk as lots of 15–25 fish each and returned alive to their respective tank. All fish were harvested from tanks on days 146–148 (11–13 October 2016). A minimum of 150 fish/tank were weighed individually (to the nearest 0.1 g) and the remainder were counted and weighed in bulk (to the nearest 0.1 kg). Individually weighed fish were assigned to size classes (g/fish): very small (VS; < 340 g), small (S; 340–453 g), medium (M; 454–567 g), large (L; 568–681 g), very large (VL; 682–795 g), and

jumbo (J; ≥795 g). Condition indices (hepatosomatic index, HSI; intraperitoneal fat, IPF; and, muscle ratio, MR) were measured on arbitrary subsets of ten euthanized fish from each 150-fish sample. Similarly, subsets of five fish from each 150-fish sample were euthanized, frozen, and analyzed later for body composition and nutrient and energy retentions.

2.4. Diet and tissue chemical analyses

Whole body initial and final fish and final muscle samples were prepared for analysis as described previously (Rawles et al., 2018) with the following differences: fish from each tank were processed and analyzed individually for whole-body analysis, whereas muscle samples were pooled by tank for homogenization and analysis.

Proximate composition of diets, fish, and tissues was determined according to standard methods (AOAC, 2006) as described previously by Rawles et al. (2018). Frozen whole livers were minced over ice and glycogen was extracted according to Hassid and Abraham (1957). Glycogen was measured using a commercial colorimetric / fluorometric assay kit (No. K646-100, BioVision, Inc., Milpitas, CA USA).

Amino acid levels were determined by high pressure liquid chromatography using a fluorescent detector (FP 2020; Jasco Inc., Easton, MD, USA) on a Jasco HPLC separation system using the AccQ-Tag® method (Waters Corp., Milford, MA, USA) with Norvaline as an internal standard. In brief, post hydrolysis was conducted with 6 N HCl at 110 °C for 22 h, amino acids were neutralized with 2 M K_2CO_3, filtered (0.2 mm IC Millex®-LG, Merck Milipor Ltd., Tullagreen, Carrigtwohill Co. Cork, Ireland), and derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, then separated on a C-18 reverse phase column (Waters Corp., Milford, MA, USA). Peaks were identified and quantified by comparing to casein to obtain a hydrolysis correction value and a known standard (Amino Acid Standard H, Thermo Scientific, Rockford, IL, USA).

Protein, energy and amino acid retention efficiencies were estimated as: protein, energy, or amino acid retention efficiency (RE) = (protein, energy, or amino gain × 100)/ (protein, energy, or amino acid fed). To relate fish performance to amino acid profile of the diet relative to the IP model, the distance between the amino acid profiles of the diets and hybrid tilapia muscle at each DP level was defined as the sum of the squared deviations (SS Dev) from the IP concentrations as described in Rawles et al. (2018).

2.5. MIB and geosmin analyses

Water samples for analysis of 2-methylisoborneol (MIB) and geosmin were collected from each tank beginning 8 d before stocking and continuing at approximately 21-d intervals until day 141. After harvest, a sample of biofilm/crust from along the water line was collected from each tank. Individually labelled 20-mL scintillation vials were filled completely with tank water or biofilm/crust and stored at 4 °C. One fillet from each of five fish per tank, collected as part of the MR analysis at harvest, was placed in an individually labelled plastic bag, vacuum sealed, and stored frozen. All samples were sent by next-day delivery for analysis at the USDA-ARS Natural Products Utilization Research Unit, University, MS USA. Analytical procedures for determination of MIB and geosmin followed Schrader et al. (2011). The instrumental detection limit for each compound was 1 part per trillion.

2.6. Statistical analyses

Normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) of data were confirmed using SAS version 9.4 (SAS Institute, Inc., Cary, NC USA). Fish production and growth, tissue composition, nutrient retention efficiencies, and water quality data were analyzed by mixed models analysis of variance (ANOVA) using PROC MIXED and linear regression analysis using PROC REG (SAS version 9.4). A mixed models repeated measures ANOVA (compound symmetry covariance structure) was used to analyze body compositional indices data, tank chlorophyll *a* and settling chamber water quality variable concentrations over time, and MIB and geosmin concentrations in fillet meat and over time in tank water. The CONTRAST statement or all-pairwise means comparison of least squares means was performed using the DIFF option with the Bonferroni adjustment of *P* values. Percent data were arcsin transformed before data analysis (Sokal and Rohlf, 1995). The FREQ procedure in SAS generated Chi-square and Likelihood Ratios that were used in the analysis of harvested fish size distributions to identify associations among diets and fish size class. Differences among responses were declared significant at $P \leq .05$.

3. Results

3.1. Fish and feed performance, and condition indices

Gross fish yield, net fish yield, weight gain, and the percent of fish larger than 454 g were significantly greater in the 27.7% DP treatment than in the 22.5% DP treatment, while the 32.3% DP treatment was intermediate (Table 3). Once feeding of test diets began, the growth curve slope for fish fed the 27.7% DP diet (3.96) was significantly greater ($P = .037$) than that for fish fed the 22.5% DP diet (3.56), while the 32.3% DP diet growth curve slope (3.85) was intermediate (Fig. 2). Average final weight was significantly smaller ($P = .041$) and FCR was significantly greater ($P = .015$) in the 22.5% DP treatment than in the other two treatments, which did not differ. Total feed nitrogen and phosphorus, liver size (HSI), and muscle ratio in the 27.7% DP treatment were intermediate to the other two treatments, which differed significantly. Daily feed ration did not differ significantly among diets ($P = .160$) and averaged 158 g/m^3, 162 g/m^3, and 154 g/m^3 for the 22.5%, 27.7%, and 32.3% DP treatments, respectively. All remaining response variables were independent of dietary digestible protein.

3.2. Diet, whole body, and muscle composition and nutrient retentions

Because of differences between tabulated and actual nutrient concentrations in the ingredients used for this study, amino acid composition of the test diets varied from formulated levels (Table 2). Concentrations of essential amino acids in the test diets exceeded requirements listed in the NRC (2011), while absolute concentrations of most amino acids in the test diets exceeded those of the IP model except for Asx, Gly and Met which fell short of IP targets. In general, both total and unsupplemented amino acid sum of squared deviations from the ideal were similar and lower for the 22.5% and 27.7% DP diets when compared to those of the 32.3% DP diet.

Whole-body proximate composition did not differ significantly among dietary treatments (Table 4). Except for Asx, Glx, Ile, and Ser, whole-body amino acids did not differ significantly with respect to diet DP.

Protein retention efficiency was similar and higher for the 22.5% and 27.7% DP diets, than for the 32.3% DP diet (Table 5). Lipid and energy retentions were not significantly affected by diet DP. Whole body retention efficiencies of most amino acids were significantly lower for the 32.3% DP diet. Diet DP did not affect whole body retention efficiencies of Ala, Arg, Gly, Lys, and Phe.

Muscle proximate composition was not significantly affected by diet DP level (Table 6). Muscle His, Ile, Leu, and Tyr in fish fed the 22.5% DP diet generally were significantly lower than for the 27.7% DP diet.

3.3. Fish size distributions

Fish market size-class distributions were associated strongly with dietary DP level as indicated by the Likelihood Ratio Chi-square statistic ($P < .001$) of the size class frequency analysis (Fig. 3). Specifically, there were more than expected fish in the very small (VS) and small (S)

Table 3
Least squares means and pooled (SE*p*) for growth, feed performance, size distribution parameters, and composition indices of hybrid tilapia, *Oreochromis aureus* × *O. niloticus*, (initial weight: 31.9 ± 0.1 g/fish) reared in outdoor biofloc production technology tanks to market size.[a]

Response[c]	DP (%)[b]			SE*p*	*ANOVA*
	22.5	27.7	32.3		*Pr* > *F*[d]
GFY	14.0 b	15.6 a	14.9 ab	0.2	0.010
NFY	13.1 b	14.7 a	14.0 ab	0.2	0.011
Survival	98.0	99.8	99.1	0.0	0.055
Total feed	19.9	20.8	19.8	0.3	0.094
Total feed N	968 c	1155 b	1247 a	18	< 0.001
Total feed P	175 b	186 ab	191 a	3	0.016
FCR	1.53 a	1.40 b	1.41 b	0.02	0.015
Gain	1478 b	1639 a	1555 ab	32	0.035
Avg wt	518 b	564 a	553 a	10	0.041
Min wt	221	185	273	25	0.114
Max wt	795	867	803	23	0.130
% > 454 g	72.8 b	83.0 a	81.5 ab	2.3	0.038
CV	22.4	20.6	18.8	1.1	0.143
Skewness	−0.14	−0.02	−0.04	0.08	0.427
Kurtosis	−0.19	0.21	−0.20	0.14	0.155
IPF	5.8	6.1	5.3	0.3	0.293
MR	44.6 b	45.4 ab	46.2 a	0.3	0.023
HSI	4.35 a	4.04 ab	3.34 a	0.00	0.023
Protein	7.46	7.63	8.90	0.36	0.095
Lipid	18.56	15.73	15.71	1.91	0.483
Glycogen	4.68	4.84	4.67	0.19	0.799
Moisture	60.81	64.03	62.38	1.40	0.302

[a] $N = 3$ replicate tanks.
[b] Percent diet digestible protein (DP).
[c] GFY: gross fish yield (kg/m^3) after 147 days; NFY: net fish yield (kg/m^3); Survival (%); Total feed (kg/m^3, dry weight basis) consumed; Total feed nitrogen (g N/m3, dry weight basis); Total feed phosphorus (g P/m^3, dry weight basis); FCR: feed conversion ratio = g dry feed consumed / g weight gained; Gain (%) = (final weight – initial weight) * 100 / initial weight; Avg wt: average fish weight (g) at harvest; Min wt: minimum fish weight (g) at harvest; Max wt: maximum fish weight (g) at harvest; % > 454 g: percent of fish weighing > 454 g at harvest; CV: coefficient of variation in fish size distributions; Skewness – denotes whether the fish size distribution is weighted toward smaller fish with fewer larger fish, i.e., right-tailed (+), heavier toward larger fish with fewer smaller fish, i.e., left-tailed (−), or symmetric (0) about the mean; Kurtosis – denotes whether the fish size distribution is flattened (−) about the mean, indicating fish are evenly distributed among size categories, or peaked (+) about the mean, indicating fish are bunched among few size categories around the mean; IPF: intraperitoneal fat (%) = intraperitoneal fat mass * 100 / fish mass; MR: muscle ratio (%) = fillet with rib mass * 100 / fish mass; HSI: hepatosomatic index (%) = liver mass × 100 / fish mass; liver protein, lipid, glycogen, and moisture (%, fresh-weight).
[d] ANOVA, *Pr* > F. LS means in the same row with different letters are different ($P \leq .05$).

size classes, and fewer than expected fish in the large (L) and jumbo (J) size classes in 22.5% DP treatment tanks. Fewer than expected VS and S fish and more than expected L and J fish were present in 27.7% DP treatment tanks. In 32.3% DP treatment tanks, there were fewer than expected fish in the VS size class and more than expected fish in the L size class.

3.4. MIB and geosmin concentrations

Aqueous concentrations of MIB and geosmin were low on all sampling dates and did not differ significantly among treatments except on day 141 when concentrations in the 27.7% DP treatment were significantly greater than in the other two treatments (Table 7).

Mean MIB concentration in fillet meat was significantly higher in the 27.7% DP treatment compared to the 32.3% DP treatment, while the 22.5% DP treatment mean did not differ significantly from the other treatment means (Table 8). No significant treatment differences were detected for fillet geosmin concentration. Concentrations of MIB and geosmin detected at harvest in the biofilm/crust along the water line in tanks did not differ significantly among treatments.

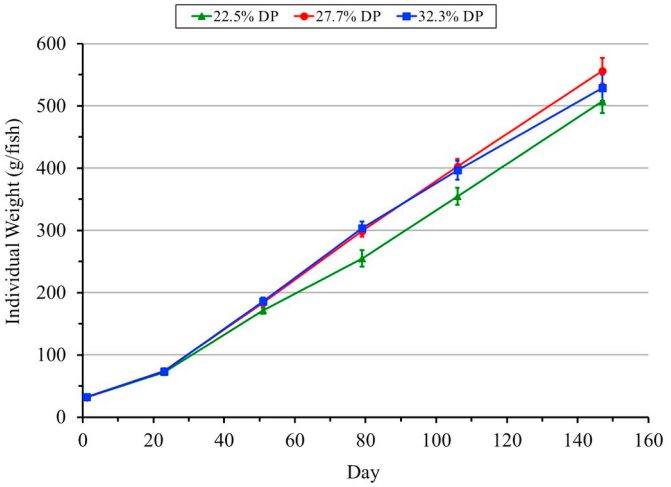

Fig. 2. Growth (mean ± SE) of hybrid tilapia in outdoor biofloc production technology tanks fed diets that contained one of three digestible protein (DP) levels.

Table 4
Least squares means and pooled SE (SE*p*) for whole body composition (fresh-weight basis) of hybrid tilapia reared in outdoor biofloc production technology tanks to market size.[a]

Response[c]	DP (%)[b]			SE*p*	*ANOVA*
	22.5	27.7	32.3		*Pr* > *F*[d]
CP	15.22	16.24	16.48	0.37	0.133
Lipid	13.79	12.42	11.93	0.60	0.211
Energy	9.25	8.92	8.84	0.23	0.570
Moisture	65.24	65.76	65.64	0.97	0.821
Ash	11.75	12.70	12.94	0.60	0.395
ALA	1.06	1.07	1.11	0.03	0.286
ARG	0.93	0.94	0.97	0.02	0.365
ASX	1.37 b	1.48 a	1.47 a	0.03	0.013
GLX	2.17 b	2.31 ab	2.33 a	0.05	0.035
GLY	1.41	1.39	1.50	0.05	0.233
HIS	0.44	0.45	0.46	0.01	0.206
ILE	0.52 b	0.56 a	0.55 ab	0.01	0.015
LEU	1.07	1.14	1.13	0.02	0.075
LYS	1.09 b	1.17 ab	1.19 a	0.03	0.037
MET	0.37	0.37	0.36	0.01	0.930
PHE	0.63	0.62	0.63	0.02	0.879
SER	0.57	0.62	0.62	0.02	0.062
THR	0.64	0.68	0.68	0.01	0.067
TYR	0.44	0.47	0.46	0.01	0.065
VAL	0.71	0.73	0.73	0.01	0.171

[a,b,d] see footnotes Table 3.
[c] Composition includes CP: crude protein (g/kg), lipid (g/kg), energy (MJ/kg), moisture (g/kg), and ash (g/kg), and amino acids (g/kg).

3.5. Tank water quality dynamics

Mean minimum daily DO concentration, as percent saturation, did not differ significantly among treatments during any month ($P = .526$) and exceeded 69% saturation. Mean daily water temperature did not differ significantly among DP treatments during any month ($P = .792$), increasing from 22.8 °C in May to 30.4 °C in July, then decreasing to 22.1 °C in October. Minimum mean daily water temperatures also did not differ significantly among treatments during any month ($P = .693$) and averaged 21.2 °C, 28.9 °C, and 20.7 °C in May, July, and October, respectively.

Table 5
Least squares means and pooled SE (SE*p*) for whole body retention efficiencies (RE, %) of protein, energy, lipid, and amino acids in hybrid tilapia reared in outdoor biofloc production technology tanks to market size.[a]

Response[c]	DP (%)[b]			SE*p*	*ANOVA*
	22.5	27.7	32.3		*Pr > F*[d]
PRE	33.4 a	33.4 a	29.6 b	0.5	0.003
Lipid	33.5	30.8	32.5	2.3	0.703
ERE	32.2	32.3	31.7	1.3	0.937
ALA	35.8	33.3	34.1	1.11	0.331
ARG	36.8	34.2	33.1	0.95	0.083
ASX	35.9 a	36.9 a	30.7 b	0.78	0.002
GLX	27.3 ab	27.8 a	24.9 b	0.63	0.038
GLY	66.8	61.1	57.6	2.58	0.103
HIS	29.8 a	27.6 ab	25.7 b	0.58	0.009
ILE	27.2 a	26.7 a	23.6 b	0.40	0.001
LEU	24.9 a	25.0 a	22.6 b	0.46	0.016
LYS	29.5	30.5	30.1	0.80	0.724
MET	47.7 a	42.2 b	41.0 b	1.16	0.015
PHE	23.6	21.1	19.9	1.30	0.212
SER	27.7 a	26.7 a	23.9 b	0.65	0.012
THR	31.4 a	29.5 ab	26.5 b	0.79	0.011
TYR	32.7 a	30.8 a	27.5 b	0.63	0.003
VAL	27.9 a	27.4 a	24.8 b	0.46	0.006

[a,b,d]see footnotes Table 3.
[c]Retention efficiencies include PRE: protein retention efficiency = g protein gain × 100/g protein fed, Lipid RE: lipid retention efficiency = g lipid gain × 100/g lipid fed, ERE: energy retention efficiency = kcal energy gain × 100/kcal energy fed, and amino acid retention efficiency = individual amino acid gain (g) × 100/g individual amino acid fed (g).

Table 6
Least squares means and pooled SE (SE*p*) for muscle proximate and amino acid composition (fresh-weight basis) of hybrid tilapia reared in outdoor biofloc production technology tanks to market size.[a]

Response[c]	DP (%)[b]			SE*p*	*ANOVA*
	22.5	27.7	32.3		*Pr > F*[d]
CP	18.74	19.46	19.13	0.15	0.274
Lipid	4.90	4.91	4.65	0.23	0.867
Energy	6.52	6.81	6.53	0.12	0.604
Moisture	74.35	73.20	73.97	0.33	0.451
Ash	6.64	6.12	6.30	0.29	0.600
ALA	1.06	1.10	1.10	0.01	0.317
ARG	0.97	0.99	1.00	0.01	0.130
ASX	1.80	1.82	1.85	0.02	0.311
GLX	2.83	2.84	2.88	0.03	0.525
GLY	0.93	0.98	0.96	0.02	0.469
HIS	0.53 b	0.56 a	0.56 ab	0.01	0.041
ILE	0.73 b	0.77 a	0.76 ab	0.01	0.051
LEU	1.43 b	1.53 a	1.51 ab	0.02	0.030
LYS	1.56	1.55	1.59	0.03	0.765
MET	0.52	0.56	0.54	0.01	0.065
PHE	0.76	0.86	0.80	0.02	0.080
SER	0.67	0.69	0.68	0.01	0.242
THR	0.87	0.89	0.90	0.01	0.193
TYR	0.60 b	0.63 ab	0.63 a	0.01	0.034
VAL	0.88	0.93	0.92	0.01	0.077

[a,b,d]see footnotes Table 3.
[c]Response as in Table 4 footnote c.

A TAN spike of similar magnitude (13.1–14.5 mg/L; $P = .427$) occurred in all treatments on 1 June (day 15). Cumulative feed nitrogen (dry matter basis) added through day 14 did not differ significantly among treatments ($P = .646$) and averaged 36.8–37.5 g N/m^3.

Significant differences were detected in mean water quality variables in response to diet digestible protein content (Table 9). Nitrite-N, nitrate-N, and SRP concentrations were significantly greater in the 32.3% DP treatment. Mean pH and total alkalinity were significantly greater in the 22.5% DP treatment. Mean TAN, chlorophyll *a*, SS, and TSS concentrations were independent of diet DP. Settling chamber operation maintained 200–600 mg/L TSS. No treatment by time significant differences were detected among treatments for chlorophyll *a* concentration.

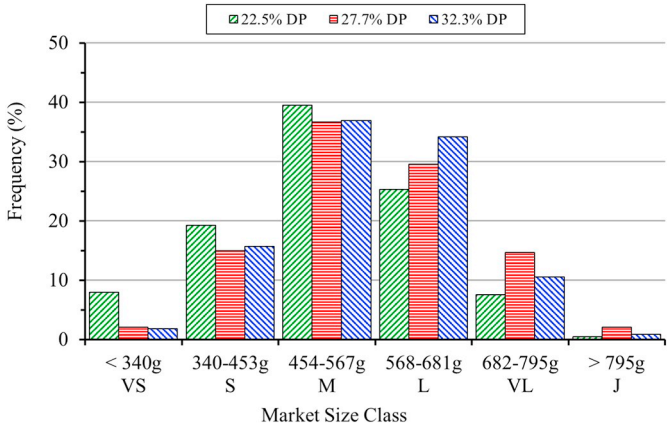

Fig. 3. Hybrid tilapia market size frequency distribution at harvest in response to diet digestible protein (DP) content. Size classes are: very small (VS), small (S), medium (M), large (L), very large (VL), and jumbo (J).

Total feed nitrogen and phosphorus added to tanks increased significantly with increased diet DP (Table 3). Mean weekly nitrate-N and SRP increased linearly ($P < .001$) with cumulative weekly feed addition within each DP treatment. Nitrate-N accumulation rate increased significantly ($P < .001$) with increased diet DP (Fig. 4), whereas SRP accumulation rate was similar ($P = .275$) among diets. Although diets were formulated to be balanced in available phosphorus, total phosphorus was slightly different by analysis, possibly because of differences in actual and digestible levels of phosphorus in individual ingredients. Significantly less ($P < .001$) sodium bicarbonate was added to 22.5% DP treatment tanks (833 g/m^3) than to 27.7% DP (1467 g/m^3) or 32.3% DP (1721 mg/m^3) tanks.

3.6. Settling chamber water quality

Settling chamber mean weekly effluent nitrate-N, chlorophyll *a*, and TSS concentrations were significantly lower than influent concentrations (Table 10). On average, 66–75% of TSS and 31–54% of chlorophyll *a* were removed from influent water by the settling chambers. Total ammonia-N concentrations in effluent were significantly greater than influent concentrations. Total alkalinity concentration in the settling chamber effluent was significantly greater ($P = .016$) than the influent concentration in the 32.3% DP treatment. No significant differences were detected for nitrite-N and SRP.

Water quality variables were transformed in the settling chamber. Net nutrient gains or losses calculated over the course of the experiment, after accounting for losses to draining, showed settling chamber effluent gained TAN, SRP, and total alkalinity, and lost nitrite-N, nitrate-N, chlorophyll *a*, and TSS (Table 11). No significant ($P > .05$) differences among diet DP were detected for any water quality variable.

4. Discussion

This is the first report that evaluates the effect of dietary protein content on hybrid tilapia productivity in a photoautotrophic-chemoautotrophic BFT system and results indicated that dietary digestible protein could be reduced from 32.3% to 27.7% with no adverse effect on tilapia production, but a reduction from 27.7% to 22.5% DP caused a significant decrease in tilapia growth and yield. Thus, only as digestible protein decreased from 32.3% to 27.7% did grazing on the biofloc by tilapia appear to substitute for dietary protein. The absence of significant treatment differences in mean chlorophyll *a* and TSS

Table 7
Least squares means and pooled SE (SE*p*) of 2-methylisoborneol (MIB, ng/L) and geosmin (ng/L) concentration over time in outdoor biofloc production technology tanks stocked with hybrid tilapia and fed for 146 days.[a]

	MIB					Geosmin				
	DP (%)[b]				*ANOVA*	DP (%)[b]				*ANOVA*
Day	22.5	27.7	32.3	SE*p*	*Pr* > *F*[c]	22.5	27.7	32.3	SE*p*	*Pr* > *F*[c]
−8	0.0	0.0	0.0	4.3	> 0.050	0.1	0.0	0.0	24.5	> 0.050
15	2.4	2.3	2.5	4.3	> 0.050	16.9	5.0	1.7	24.5	> 0.050
43	0.5	0.8	0.5	4.3	> 0.050	14.9	3.9	1.5	24.5	> 0.050
64	0.4	4.3	1.5	4.3	> 0.050	10.7	27.6	3.8	24.5	> 0.050
85	18.7	11.1	14.0	4.3	> 0.050	13.2	10.2	32.9	24.5	> 0.050
106	13.9	12.5	12.9	4.3	> 0.050	7.3	7.5	25.5	24.5	> 0.050
120	13.8	19.7	21.4	4.3	> 0.050	24.7	9.0	13.8	24.5	> 0.050
141	5.9 b	47.3 a	6.8 b	4.3	< 0.001	44.9 b	211.1 a	10.0 b	24.5	0.006

[a] N = 3 replicate tanks.
[b] Percent diet intact digestible protein (DP).
[c] ANOVA, *Pr* > F. LS means within compound in the same row with different letters are different ($P \leq .05$).

Table 8
Least squares means and pooled SE (SE*p*) of 2-methylisoborneol (MIB) and geosmin concentration at harvest in fillet meat (ng/kg) from hybrid tilapia and in the biofilm/crust (ng/L) at the water line from outdoor biofloc technology production tanks.[a]

Response	DP (%)[b]				*ANOVA*
	22.5	27.7	32.3	SE*p*	*Pr* > *F*[c]
Fillet MIB	166.6 ab	623.8 a	53.9 b	105.4	0.019
Fillet geosmin	1311.8	2174.3	918.1	617.4	0.397
Biofilm/crust MIB	19.7	783.7	91.3	432.4	0.437
Biofilm/crust geosmin	82.0	682.7	861.0	469.9	0.510

[a,b,c]see footnotes Table 7.

Table 9
Least squares means and pooled SE (SE*p*) for water quality variables in outdoor biofloc production technology tanks used to grow hybrid tilapia to market size.[a]

Response[c]	DP (%)[b]			SE*p*	*ANOVA*
	22.5	27.7	32.3		*Pr* > *F*[d]
TAN	1.08	1.06	1.64	0.17	0.092
Nitrite-N	6.88 b	6.98 b	10.70 a	0.54	0.004
Nitrate-N	71.01 c	102.73 b	129.29 a	2.42	< 0.001
SRP	17.03 b	17.28 b	20.03 a	0.51	0.010
pH	7.64 a	7.52 b	7.47 b	0.02	0.002
T Alk	81.56 a	75.29 b	71.12 b	2.18	0.039
Chl *a*	1039	1104	1264	54	0.061
SS	21	17	18	2	0.347
TSS	294.5	318.0	314.2	13.3	0.456

[a,b,d]see footnotes Table 3.
[c]Total ammonia-nitrogen (TAN; mg NH_4-N/L); nitrite-nitrogen (mg NO_2-N/L); nitrate-nitrogen (mg NO_3-N/L); soluble reactive phosphorus (SRP; mg PO_4-P/L); total alkalinity (T Alk; mg/L as $CaCO_3$); chlorophyll *a* (Chl *a*; mg/m^3); settleable solids (SS; mL/L); total suspended solids (TSS; mg/L).

concentrations suggests that biofloc quality and quantity was similar among treatments, which when considered along with the similar quantities of feed fed among treatments suggests that available nutrition in the 22.5% DP treatment may be insufficient to sustain fast growth.

Excreted feed nitrogen is assimilated by bacterial biomass in a biofloc system predominated by heterotrophic bacteria and supplemented with allocthonous organic carbon. Alternatively, in a biofloc system where photoautotrophs and chemoautotrophs are dominant, excreted feed nitrogen is used for biosynthesis by the former and primarily as an energy source by the latter. Both autotrophic and heterotrophic production contribute to fish growth (Schroeder, 1978; Hargreaves, 2006), but more biofloc system research has addressed the contribution of heterotrophic production to fish growth and for substitution of dietary protein.

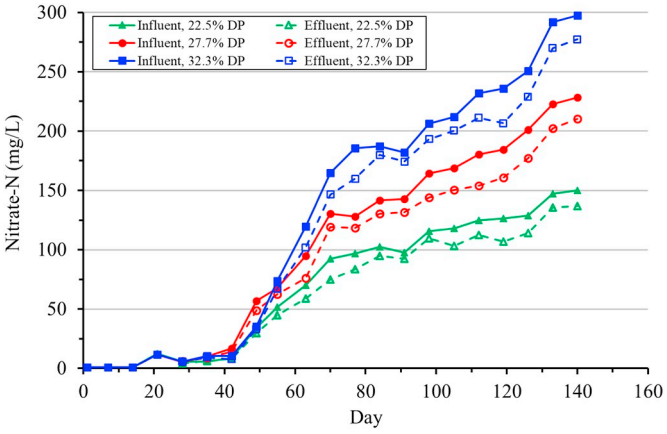

Fig. 4. Settling chamber influent (solid line/symbol) and effluent (dashed line/open symbol) nitrate-snitrogen (NH_4-N) concentrations for outdoor biofloc technology production tanks where hybrid tilapia were fed diets that contained one of three digestible protein (DP) levels. Influent concentration is the same as culture tank water concentration. Continuous operation of settling chambers began on day 22.

Biofloc derived from heterotrophic bacteria contains on a dry matter basis 25% protein and 7.2% lipid, and while its essential amino acid profile indicates it is a useful protein source for tilapia, Met, Arg, and Lys are relatively deficient (Ekasari et al., 2014). However, biofloc management protocols, i.e., exogenous organic carbon source and target carbon:nitrogen ratio, affect biofloc protein content as indicated by reported crude protein (dry matter basis) values that range from 14.2% to 41.2% (Azim and Little, 2008; Wei et al., 2016; Luo et al., 2017; da Silva et al., 2018). Tilapia consume biofloc readily and retain 24–32% of biofloc nitrogen, suggesting that biofloc can substitute for a portion of the daily ration of formulated feed (Avnimelech and Kochba, 2009; Ekasari et al., 2014).

However, the degree to which biofloc can substitute for formulated feed protein appears to vary with life stage. The only two published studies that we found on dietary protein reduction in BFT systems addressed growth of fingerling (10 to 60 g/fish) and juvenile or stocker (60 to 230 g/fish or 100 to 140 g/fish) tilapia (Azim and Little, 2008; da Silva et al., 2018). Their results showed that grazing on the biofloc would reduce crude protein of formulated feed from 33% to 28% for fingerlings and from 33% to 35% to 22–24% for juvenile tilapia, whereas we found that formulated feed digestible protein could be

Table 10
Least squares means and SE of the difference (SE*d*) for water quality variable concentrations for influent and effluent from two 117-L settling chambers in series connected to outdoor biofloc production technology tanks used to grow hybrid tilapia to market size.[a]

DP (%)[b]	Response[c]						
	TAN	Nitrite-N	Nitrate-N	SRP	T Alk	Chl a	TSS
22.5							
Influent	0.33	7.10	86.83	19.7	82.95	1040	294.5
Effluent	1.08	6.82	77.41	20.39	98.00	526	81.3
SE*d*	0.11	0.30	1.70	0.27	3.25	82	17.2
ANOVA Pr > F[d]	0.008	1.000	0.022	0.688	0.053	< 0.001	< 0.001
27.7							
Influent	0.34	7.32	126.06	20.32	73.99	1104	318.0
Effluent	0.95	7.04	112.66	21.11	85.67	503	76.8
SE*d*	0.11	0.30	1.70	0.27	3.25	82	17.2
ANOVA Pr > F	0.023	1.000	0.003	0.417	0.171	< 0.001	< 0.001
32.3							
Influent	0.89	11.88	158.88	23.15	69.01	1264	314.2
Effluent	1.58	11.42	145.51	23.61	88.09	867	108.1
SE*d*	0.11	0.30	1.70	0.27	3.25	82	17.2
ANOVA Pr > F	0.012	1.000	0.003	1.000	0.016	< 0.001	< 0.001

[a,b,d]see footnotes Table 3.
[c]see footnote Table 9.

Table 11
Least squares means and pooled SE (SE*p*) for sum of calculated gain or reduction (loss) in water quality variable mass following passage through two 117-L settling chambers in series that were connected to outdoor biofloc production technology tanks used to grow hybrid tilapia to market size.[a]

Response[c]	DP (%)[b]			SE*p*	*ANOVA*
	22.5	27.7	32.3		*Pr > F*[d]
TAN Gain	331.3	262.1	303.4	50.4	0.643
Nitrite-N Reduction	47.2	90.6	120.3	104.5	0.886
Nitrate-N Reduction	3977.3	5690.9	5505.9	737.2	0.273
SRP Gain	367.9	428.1	285.6	134.3	0.763
T Alk Gain	190.7	147.7	208.4	30.5	0.407
Chl *a* Reduction	202.9	223.6	211.8	44.7	0.948
TSS Reduction	65,741	94,183	85,968	15,517	0.459

[a,b,d]see footnotes Table 3.
[c]Total ammonia-nitrogen (TAN; g NH_4-N); nitrite-nitrogen (g NO_2-N); nitrate-nitrogen (g NO_3-N); soluble reactive phosphorus (g PO_4-P); total alkalinity (T Alk; equivalents as $CaCO_3$); chlorophyll *a* (g Chl *a*); total suspended solids (g TSS).

reduced 32.3% to 27.7% when growing tilapia to market size. The TSS concentration and stocking rate in the present study were lower than in the above-cited studies. While these two factors (tilapia life stage and TSS concentration) make direct comparison among the studies difficult, some differences are worth noting. Higher solids concentrations, 1150–1323 mg TSS/L (da Silva et al., 2018) and ca. 580–1000 mg TSS/L (Azim and Little, 2008), indicated a more abundant biofloc (natural food source) that the smaller fish stocked in these studies likely were able to consume more efficiently. Moreover, Zhao et al. (2016) found that addition of organic carbon increased and enhanced the nutritive value of the biofloc. Fish grew more slowly in these studies than in the present study potentially because stocking rates were 80 fish/m^3 (da Silva et al., 2018) and 100 fish/m^3 (Azim and Little, 2008), compared to 29 fish/m^3 in the present study. Growth of tilapia in the BFT system at HKDSNARC from 60 g/fish to 250 g/fish was 2.2 g/d (83 fish/m^3) and from 100 g/fish to 140 g/fish was 1.9 g/d (112 fish/m^3) (Green, B.W., unpublished data), comparable to da Silva et al. (2018), but higher than Azim and Little (2008), who stocked mixed-sex tilapia. Fish yields are higher when smaller fish are grown because despite lower absolute growth by individual fish, higher numbers result in greater yields (Hepher, 1978), and this was exemplified by da Silva et al. (2018) where gross fish yield for fish grown from 10 to 60 g/fish (400 fish/m^3) ranged from 16.4–24.9 kg/m^3 and for fish grown from 60 to 250 g/fish (80 fish/m^3) ranged from 14.9–19.5 kg/m^3.

A number of studies evaluated the effect of dietary protein level on tilapia performance, but few are relevant to the current study because small fish were grown for short periods (Twibell and Brown, 1998; Furuya et al., 2005; Abdel-Tawwab and Ahmad, 2009; Abdel-Tawwab et al., 2010; Kpundeh et al., 2015; Ma et al., 2015; Ye et al., 2016; Liu et al., 2018c) or advanced fingerlings were grown to stocker size (200–250 g/fish) (El-Saidy and Gaber, 2005; Sweilum et al., 2005; Botaro et al., 2007). Only one published study was found where tilapia (10 fish/m^3) were grown in RAS from 100 to about 472 g/fish and fed diets (22.5–26.7% digestible protein) formulated using ideal protein theory and supplemented with Lys, Arg, Met, and Thr (Righetti et al., 2011). Results showed digestible dietary protein could be reduced from 26.75% to 24.5% based on the quadratic response of FCR to digestible dietary protein level.

In the current study, FCR became slightly poorer (increased) at the lowest DP in market size tilapia. Hepatosomatic index (HSI), typically a sensitive indicator of diet nutrient balance, increased slightly with decreasing DP in the current study, whereas body fat (IPF) was unaffected by the three levels of DP tested. Furuya et al. (2005) and Abdel-Tawwab et al. (2010) also found increasing HSI with decreasing DP in juveniles. However, Furuya et al. (2005) saw no trends in FCR or IPF in juveniles up to 125 g/fish, whereas Abdel-Tawwab et al. (2010) also observed poorer FCR at the lowest level of diet protein (25%). Botaro et al. (2007) found no change in FCR from 22.7 to 27% DP in juveniles up to 250–270 g/fish average weight but saw the opposite trend in HSI from 1.86% at 27% DP to 1.66% at 24.3% DP; however, HSI increased to almost 2% at the lowest (22.7%) DP.

Retention efficiencies of protein (Furuya et al., 2005; Li et al., 2013) and amino acids (Botaro et al., 2007) in tilapia tend to increase, while tissue levels of protein (Azim and Little, 2008; Abdel-Tawwab et al., 2010) and hence amino acids tend to decrease in fish with decreasing DP in the diet, as seen in the current study. Differences in amino acids composition of muscle and whole-body were seen primarily in decreased concentration at the lowest of the three tested DP levels, albeit the magnitude of the changes was small (< 0.25%) but statistically significant. This response in combination with the lack of significant

differences in muscle and whole-body proximate composition suggest all three test diets were balanced sufficiently to meet basic metabolic needs but insufficient to meet maximum performance potential at the lowest tested DP. The lack of strong trends in nutrient depots within the liver as diet DP level changed further corroborates the idea that diets were well balanced. Specifically, liver protein and glycogen were relatively flat at slightly < 9% and 5%, respectively, while liver lipid numerically increased about three percentage points only at the lowest diet DP level (from 15.7% to 18.5%), although the increase was not close to significant ($P = .483$). Liver moisture also varied about four percentage points among fish fed the different diets, but those differences also were not significant ($P = .302$).

In terms of comparisons of tissue compositions in response to dietary protein level in tilapia there are no examples in the literature available for market-size fish. However, similar to our results, Abdel-Tawwab et al. (2010) also saw a tendency for increasing protein retention efficiency, decreasing whole body protein, and increasing whole body lipid when diet protein decreased from 45% to 25% in fry to 60-g juveniles produced in BFT. In a short 12-week study, Azim and Little (2008) grew tilapia from about 100 g/fish to 140 g/fish in a light-limited BFT system at two levels of diet CP (35% vs 25%) and also saw numerical decreases in whole body protein (53% to 49%) and increases in whole body lipid (28% to 33%) with decreasing diet CP, though those trends were not statistically significant.

While gains in overall protein retention were modest in the current study—from approximately 30% at 32.3% DP to 33% at 27.7—22.5% DP, larger increases were seen in the retention of individual amino acids that exceeded 8 percentage points in some instances. Interestingly, among the four supplemented amino acids, Lys retention (≈ 30%) and Lys muscle concentrations (≈ 1.6%) were completely unaltered by diet DP level. This suggests that diet levels of Lys may have been first limiting. The drop in whole body Lys at the lowest DP level (from 1.19%–1.17% to 1.09%) as well as similar drops seen in whole body levels of several essential amino acids at the lowest diet DP substantiates the inadequacy of the 22.5% DP diet for maximum performance of Nile tilapia in BFT. All relevant studies conducted in tilapia thus far have found that 22.5% DP is too low for optimum production, whereas about 25%–27% DP appears appropriate for fast growing tilapia to market size if careful attention is paid to diet formulation and feeding. The one exception is da Silva et al. (2018) who concluded that dietary CP could be lowered to 22%, which corresponded to 20% DP, in tilapia grown in BFT from 60 to 230 g/fish, based on a fit of the linear response plateau (LRP) model to weight gain (see da Silva et al., 2018, Fig. 3b). In that study, only linear or quadratic models were examined, but a closer look at the weight gain and other response plots confirms their data are not quadratic. However, LRP, i.e., broken-line models, tend to underestimate nutrient requirements (Shearer, 2000; NRC, 2011).

He et al. (2013) showed that maintenance needs for amino acids increase with tilapia size while amino acid utilization efficiency for gain above maintenance decreases with increasing fish size. According to their estimate 152 mg of digestible Lys intake was required in adult tilapia for 1 g protein deposition, whereas only 101 mg of digestible Lys intake was required in juvenile tilapia for the same protein gain. Assuming that the formulated digestible Lys values are relatively accurate in the current study, the 22.5% DP diet would be expected to provide 18.9 g digestible Lys/kg diet (18.9 mg/g) while the 27.7% DP diet would provide 23.6 g/kg diet (23.6 mg/g). Hence, fish consuming the 22.5% DP diet would need to consume 152/18.9 = 8 g feed/day and fish consuming the 27.7% DP diet would only need to consume 152/23.6 = 6.4 g feed/day to achieve the same protein gain. This equates to 1.8% and 1.4% feed intake/body weight/day, respectively, for market-size fish (454 g/fish) to achieve the same protein gain. These feeding rates correspond well with the results of a bioenergetics approach by Chowdhury et al. (2013) who found that the optimum feed rates were 1.9% and 1.2% of body weight for 400-g tilapia fed diets containing 22% and 25% DP and 13 MJ/kg and 13.9 MJ/kg digestible energy (see Chowdhury et al. 2013, Table 3, *Growout*), respectively. Reduced protein diets in the current study were formulated to contain 22.5% and 27.7% DP and 13.4 MJ/kg digestible energy. Hence, given that intake, PRE, and Lys retention were not different between the two reduced DP diets, it is not surprising that the 22.5% DP diet could not approach the gain of the 27.7% DP diet over the same period of growth and fairly constant conditions. On the other hand, PRE and retention efficiencies of most of the measured amino acids were significantly poorer at the highest, 32.3%, DP diet. This in combination with the poorer production metrics in fish fed the 32.3% confirms the inefficiency of that diet formula for growing market-size tilapia in BFT systems. Interestingly, Trung et al. (2011) suggested the optimum DE and DP for growing tilapia from 100 to 500 g/fish at 28 °C to be 14 MJ/kg and 36.8% to 31.7% DP, respectively, at feed intakes of 2.1% to 1.1% of body weight, respectively, based on a bioenergetic factorial modelling approach. The latter work, however, is based on flow-through freshwater tank studies and does not account for supplemental nutrition derived from the biofloc.

The question remains as to whether further reduction in protein, between 27.7% and 22.5% DP, is possible for production of market-size tilapia in the current or similarly managed BFT systems. Furuya et al. (2012) point out that current DP in pond and net production diets for tilapia has been reduced from 26.7% to 24.6% in Brazil. First, the matrix of ingredients typically used in Brazil results in the need to supplement Lys, Met, Thr, and Arg on an ideal basis (Furuya et al., 2012; Table 2). For the matrix of ingredients used in the current study we found Ile fourth-limiting as opposed to Arg. Moreover, Arg concentrations in whole body and muscle did not vary markedly (≤ 0.04%) in response to the dietary treatments of our study. Secondly, we targeted the ideal protein concentrations for limiting amino acids at each level of reduced DP, rather than a higher level of DP as in Furuya et al. (2005) and Botaro et al. (2007).

Tilapia tainted by MIB and geosmin have been reported for fish produced in pond culture (Noomhorm and Yamprayoon, 2000; Podduturi et al., 2017) and in RAS (Guttman and van Rijn, 2008), but no previous reports were found for tilapia produced in the biofloc system. Tank aqueous MIB and geosmin dynamics in the present study were similar to those observed in earlier studies on channel catfish in our biofloc production system (Schrader et al., 2011; Green et al., 2014). The highest mean concentration of MIB (47.3 ng/L) and geosmin (211.1 ng/L), observed in the 27.7% DP treatment on day 141, was lower than > 700 ng/L MIB and > 2000 ng/L geosmin that can occur in earthen catfish pond water in the southern USA (Schrader and Blevins, 1993; Schrader and Dennis, 2005; Zimba and Grimm, 2003). Despite the low aqueous concentrations of MIB and geosmin, fish bioaccumulated sufficient quantities of both in their flesh at significantly higher concentrations. Detection threshold levels for geosmin and MIB can vary for each compound and among fish species [e.g., sensory detection thresholds reported for channel catfish (*Ictalurus punctatus*) for MIB of 100–200 ng/kg and for geosmin of 250–500 ng/kg (Grimm et al., 2004); sensory detection threshold reported for geosmin in rainbow trout (*Oncorhynchus mykiss*) was slightly < 100 ng/kg (Petersen et al., 2011)]. Without established threshold detection values for these compounds in tilapia flesh and because there was not any sensory evaluation performed on the fillets in this study, it is not certain if the MIB and geosmin concentrations in the tilapia flesh would have resulted in the fillets being classified as off-flavor. The cause of the significantly higher MIB concentration and numerically higher geosmin concentration in the 27.7% DP treatment is unknown. Bioaccumulation by fish of MIB and geosmin, through gill and gut uptake, is dependent on compound concentration and water temperature, and varies among individual fish depending upon whole-body lipid content (Howgate, 2004). Coefficients of variation for MIB and geosmin concentrations in fillets from fish within tanks ranged from 19% to 102% and 10–68%, respectively, despite no significant treatment differences in whole-body

lipid content. The lack of correlation of off-flavor compound concentration with lipid content indicates that uptake via the gut, possibly due to differences in feeding by individual fish on the biofilm crusts of the tanks, may be the reason for the variations in MIB and geosmin concentrations in the fish flesh.

Certainly, waterborne MIB and geosmin were taken up via passive diffusion across the gills by tilapia in the present experiment, but the low and temporally variable concentrations observed in treatment tanks suggest an additional source of these compounds. Higher, but variable, concentrations of MIB and geosmin were detected in the sample of water line biofilm/crust (a form of periphyton) collected after harvest. Although accumulated biofilm/crust was cleaned weekly, it re-established quickly because of the high daily feed applications. We attempted unsuccessfully to isolate the microorganism(s) producing the off-flavor compounds. Despite being fed to apparent satiation at least once daily, some tilapia were seen grazing on the biofilm/crust whenever it was present and accessible. Tilapia consume periphyton readily in the presence or absence of formulated feed (Azim et al., 2003; Milstein et al., 2008). Thus, it seems likely that biofilm/crust-derived MIB and geosmin, even if concentrations varied temporally, contributed to their bioaccumulation in tilapia flesh in the present study. In our channel catfish studies, where concentrations and temporal variation of aqueous MIB and geosmin were like those in the present study, fish in only 11–22% of tanks would be classified as off-flavor based on fillet MIB or geosmin concentrations (Schrader et al., 2011; Green et al., 2014). In those studies, channel catfish, unlike tilapia, were not observed grazing on the biofilm/crust along the tank water line. Given the putative contribution of the biofilm/crust to MIB and geosmin bioaccumulation, a suggested good management practice would be to set a cleaning frequency that minimizes development of the water line biofilm/crust. In addition, further efforts are needed to identify the source microorganisms so effective control measures can be developed.

Onset of nitrification in the present experiment followed the typical pattern observed when starting up a new biofloc (Luo et al., 2013), which was the same in all treatments because fish initially were fed similar quantities of the same feed. The TAN spike was similar in magnitude and occurred contemporaneously in all treatments after fish were fed similar quantities of feed nitrogen from the initial commercial diet. Effect of dietary protein on nitrification (as indicated by nitrate accumulation) was manifested clearly by the positive linear relationship between nitrate-N accumulation rate and dietary protein, and was supported by the significantly greater quantities of sodium bicarbonate added to the 27.7% and 32.3% DP tanks. Since alkalinity is consumed during nitrification, a source of alkalinity, sodium bicarbonate in the present experiment, is needed to maintain an optimal pH range (7.5–8.0) (Tchobanoglous et al., 2003). Although the common stocking rate and initial feed used in the present experiment resulted in simultaneous onset of nitrification, the effect of dietary protein likely would have been observed differentially had test diets been fed beginning at stock out. Feed nitrogen addition at nitrification onset in a channel catfish biofloc production system did not differ significantly among stocking rates (grand mean = 27.4 g N/m^3) but required significantly more feed days to attain at the low compared to the high stocking rate (Green, 2010). Thus, it appears that in an outdoor biofloc system dominated by photoautotrophic and chemoautotrophic processes onset of nitrification may occur once feed nitrogen addition exceeds about 30 g N/m^3.

Phosphorus concentration in tank water was related directly to feed phosphorus input. Since tilapia retain 29–38% of dietary phosphorus (Cao et al., 2008; Lu et al., 2009), excreted phosphorus accumulated in the BFT system. Except for losses to draining of the settling chambers, phosphorus is cycled through inorganic (soluble reactive phosphorus) and organic forms (living and dead). Phosphorus is assimilated by algae and bacteria under aerobic conditions and mineralized by bacteria under anaerobic conditions (van Rijn et al., 2006).

Operation of settling chambers maintained TSS concentration within a consistent range that did not appear to affect tilapia performance adversely in the present experiment. When solids removal is minimal or intermittent in a BFT system TSS concentration exceeded 1000 mg/L but did not affect negatively tilapia growth or yield (Azim and Little, 2008; Long et al., 2015; Liu et al., 2018d), and mean TSS concentrations as high as ca. 2000 mg/L did not affect growth or yield of fingerling or juvenile tilapia (Perez-Fuentes et al., 2016; da Silva et al., 2018). However, 400–500 mg/L TSS appears to be a common target concentration for tilapia (Azim and Little, 2008; Long et al., 2015; Liu et al., 2018a, 2018b).

In addition to reducing influent water TSS concentration, passage through the settling chambers changed other nutrient concentrations. The observed net gains and losses in mass of water quality variables throughout the experiment suggests that multiple anaerobic processes (transformations) occurred. The gain in total alkalinity and loss in nitrate-N suggested that nitrate-N was denitrified. Based on the production of 3.57 g of alkalinity per gram of nitrate-N reduced (Tchobanoglous et al., 2003), 36–67% of the observed reduction in nitrate-N mass in the present experiment could be explained by denitrification and would represent 25–30% of total feed nitrogen fed. Influent water TSS, comprised of particulate organic matter, fish feces, phytoplankton, and occasional waste feed, served as the organic carbon source for denitrification. Floc particles in aerated activated sludge systems can have anoxic cores where denitrification occurs (Schramm et al., 1999; Satoh et al., 2003) – floc particles in the BFT system can behave similarly and serve as a source of denitrifiers in the settling chambers. The cause of the higher transformation of nitrate-N observed in the settling chamber later in the season is unknown.

Another anaerobic, bacterially mediated nitrogen transformation is dissimilatory nitrate reduction to ammonium (DNRA), where nitrate is reduced to nitrite and nitrite is reduced to ammonium and that may contribute substantially to nitrogen cycling (Burgin and Hamilton, 2007; van den Berg et al., 2016;). Concurrent DNRA and denitrification was observed in sludge from a freshwater tilapia biofloc system (Chutivisut et al., 2014), although DNRA is reported to occur preferentially when labile organic carbon concentration is high and nitrate-N concentration is low (Burgin and Hamilton, 2007). The observed gain in TAN in settling chamber effluent supports the hypothesis of DNRA activity. On the other hand, ammonification of organic matter (TSS) in the settling chamber could explain the TAN gain observed in settling chamber effluent. The fate of 33–60% or 25–59% of the observed nitrate-N reduction remained unexplained if the TAN gain resulted from ammonification or DNRA, respectively. It is likely that some nitrate-N was assimilated into bacterial biomass.

Despite the nutrient transformations that occurred in the settling chamber, nitrogen and phosphorus concentrations in the BFT system remained high because of nutrient cycling. More research is needed to optimize nitrogen (denitrification) and phosphorus (biological) removal to minimize environmental impact of discharge from the system. Refinements in diet formulation using ideal protein theory and incorporation of enzymes that improve nutrient retention also are needed.

In conclusion, apparent grazing of biofloc by hybrid tilapia appeared to allow diet digestible protein to be reduced to 27.7% from 32.3% with no adverse effects on fish production. However, grazing the biofloc did not compensate for further reducing diet DP to 22.5%, which decreased tilapia production significantly and shifted fish to smaller size classes. Fish fed the 22.5% DP diet converted feed less efficiently but retained protein as efficiently as those fed the 27.7% DP diet, whereas the lower PRE for fish fed the 32.2% DP diet suggests excessive dietary protein content. Tilapia grazed on the water line biofilm/crust, which contained high concentrations of MIB and geosmin, and was the suspected main source of these off-flavor compounds in the fish flesh. Effective measures are needed to control sources of MIB and geosmin for viable tilapia production in the BFT system. Accumulation of nitrogen and phosphorus in tank water was related to feed protein and phosphorus content and high feeding rate.

Stable concentrations of solids and phytoplankton were maintained using side-stream settling chambers. Nitrate transformation in settling chambers was by denitrification, which appeared to account for 25–30% of total feed nitrogen, and by DNRA. However, nutrient cycling between the culture tank and settling chambers resulted in sustained high nitrogen and phosphorus concentrations. Further refinements are needed to improve retention of dietary nutrients and to optimize processes for nitrogen and phosphorus removal from the BFT production system.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2019.01.034.

References

Abdel-Tawwab, M., Ahmad, M.H., 2009. Effect of dietary protein regime during the growing period on growth performance, feed utilization and whole-body chemical composition of Nile tilapia, *Oreochromis niloticus* (L.). Aquac. Res. 40, 1532–1537.

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E., Shalaby, A.M.E., 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture 298, 267–274.

AOAC (Association of Official Analytical Chemists), 2006. AOAC Official Methods, 18th ed. Association of Official Analytical Chemists, Incorporated, Arlington.

Avnimelech, Y., 1999. Carbon/nitrogen ratios as a control element in aquaculture systems. Aquaculture 176, 227–235.

Avnimelech, Y., 2007. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture 264, 140–147.

Avnimelech, Y., Kochba, M., 2009. Evaluation of nitrogen uptake and excretion by *tilapia* in bio floc tanks, using ^{15}N tracing. Aquaculture 287, 163–168.

Avnimelech, Y., Mokady, S., Schroeder, G.L., 1989. Circulated ponds as efficient bioreactors for single cell protein production. Isr. J. Aquac. 41, 58–66.

Avnimelech, Y., Diab, S., Kochba, M., Mokady, S., 1992. Control and utilization of inorganic nitrogen in fish culture ponds. Aquac. Fish. Manag. 23, 421–430.

Azim, M.E., Little, D.C., 2008. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283, 29–35.

Azim, M.E., Verdegem, M.C.J., Mantingh, I., van Dam, A.A., Beveridge, M.C.M., 2003. Ingestion and utilization of periphyton grown on artificial substrates by Nile tilapia, *Oreochromis niloticus* L. Aquac. Res. 34, 85–92.

Bomfim, M.A.D., Lanna, E.A.T., Donzele, J.L., Abreu, M.L.T., Ribeiro, F.B., Quadros, M., 2008. Reduction of crude protein with amino acid supplementation, based on ideal protein concept, in diets for Nile tilapia fingerlings. R. Bras. Zootec. 37, 1713–1720 (in Portuguese).

Botaro, D., Furuya, W.M., Silva, L.C.R., dos Santos, L.D., Silva, T.S.D., dos Santos, V.G., 2007. Dietary protein reduction based on ideal protein concept for Nile tilapia (*Oreochromis niloticus*) cultured in net pens. R. Bras. Zootec. 36, 517–525 (in Portuguese).

Burgin, A.J., Hamilton, S.K., 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front. Ecol. Environ. 5, 89–96.

Caipang, C.M., Choo, H.X., Bai, Z., Huang, H., Lay-Yag, C.M., Lim, J., 2015. Small-scale production of biofloc using various carbon sources for the freshwater culture of tilapia, *Oreochromis* sp. ABAH Bioflux 7, 103–111.

Cao, L., Yang, Y., Wang, W.M., Yakupitiyage, A., Yuan, D.R., Diana, J.S., 2008. Effects of pretreatment with microbial phytase on phosphorus utilization and growth performance of Nile tilapia (*Oreochromis niloticus*). Aquac. Nutr. 14, 99–109.

Chowdhury, M.A.K., Siddiqui, S., Hua, K., Bureau, D.P., 2013. Bioenergetics-based factorial model to determine feed requirement and waste output of tilapia produced under commercial conditions. Aquaculture 410-411, 138–147.

Chutivisut, P., Pungrasmi, W., Powtongsook, S., 2014. Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) activities in freshwater sludge and biofloc from Nile tilapia aquaculture systems. J. Water Environ. Technol. 12, 347–356.

da Silva, M.A., Alvarenga, E.R., O Alves, G.F., Manduca, L.G., Turra, E.M., Brito, T.S., Sales, S.C.M., Silva Junior, A.F., Borges, W.J.M., Teixeira, E.A., 2018. Crude protein levels in diets for two growth stages of Nile tilapia (*Oreochromis niloticus*) in a biofloc system. Aquac. Res. 49, 2693–2703.

Ekasari, J., Deasy, A., 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.

El-Saidy, D.M.S.D., Gaber, M.M.A., 2005. Effect of dietary protein levels and feeding rates on growth performance, production traits, and body composition of Nile tilapia, *Orechromis niloticus* (L.) cultured in concrete tanks. Aquac. Res. 36, 163–171.

Furuya, W.M., Pezzato, L.E., Barros, M.M., Pezzato, A.C., Furuya, V.R.B., Miranda, E.C., 2004. Use of ideal protein concept for precision formulation of amino acid levels in fish-meal-free diets for juvenile Nile tilapia (*Oreochromis niloticus* L.). Aquac. Res. 35, 1110–1116.

Furuya, W.M., Botaro, D., de Macedo, R.M.G., dos Santos, V.G., Silva, T.D., Furuya, V.R.B., Sales, P.J.P., 2005. Ideal protein concept for dietary protein reduction of juvenile Nile tilapia (*Oreochromis niloticus*). R. Bras. Zootec. 34, 1433–1441 (in Portuguese).

Furuya, W.M., Pezzato, L.E., Barros, M.M., Boscolo, W.R., Cyrino, J.E.P., Furuya, V.R.B., Feiden, A., 2010. Tabelas Brasileiras Para a Nutrição de Tilápias [Brazilian Tables of Tilapia Nutrition]. GMF, Toledo.

Furuya, W.M., Furuya, V.R.B., Nagae, M.Y., Craciano, T.S., Michelato, M., Xavier, T.O., Vidal, L.V., 2012. Nutrição de Tilápias no Brasil [Tilapia nutrition in Brazil]. Scientia Agraria Paranaemis 11, 19–34.

Gaylord, T.G., Rawles, S.D., 2005. The modification off poultry by-product meal for use in hybrid striped bass *Morone chrysops*×*M. saxatilis* diets. J. World Aquacult. Soc. 36, 365–376.

Green, B.W., 2010. Effect of channel catfish stocking rate on yield and water quality in an intensive, mixed suspended-growth production system. N. Am. J. Aquac. 72, 97–106.

Green, B.W., Schrader, K.K., Perschbacher, P., 2014. Effect of stocking biomass on solids, phytoplankton communities, common off-flavors, and production parameters in channel catfish biofloc technology production system. Aquac. Res. 45, 1442–1458.

Grimm, C.C., Lloyd, S., Zimba, P.V., 2004. Instrumental versus sensory detection of off-flavors in farm-raised channel catfish. Aquaculture 236, 309–319.

Guttman, L., van Rijn, J., 2008. Identification of conditions underlying production of geosmin and 2-methylisoborneol in a recirculating system. Aquaculture 279, 85–91.

Hargreaves, J.A., 2006. Photosynthetic suspended-growth systems in aquaculture. Aquac. Eng. 34, 344–363.

Hassid, W.L., Abraham, S., 1957. Determination of glycogen with Anthrone Reagent. In: Colowick, S.P., Kaplan, H.O. (Eds.), Methods in Enzymology. Vol. 3 Academic Press, New York.

He, Y.-Y., Tian, L.-X., Lemme, A., Gao, W., Yang, H.-J., Niu, J., Liang, G.-Y., Chen, P.-F., Liu, Y.-J., 2013. Methionine and lysine requirements for maintenance and efficiency of utilization for growth of two sizes of tilapia (*Oreochromis niloticus*). Aquac. Nutr. 19, 629–640.

Hepher, B., 1978. Ecological aspects of warm-water fishpond management. In: Gerking, S.D. (Ed.), Ecology of Freshwater Fish Production. Wiley-Blackwell, Hoboken, pp. 447–468.

Howgate, P., 2004. Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: a review of sensory aspects and of uptake/depuration. Aquaculture 234, 155–181.

Kpundeh, M.D., Xu, P., Qiang, J., He, J., Yang, H., 2015. Effects of dietary protein levels on growth performance and haemato-immunological parameters of juvenile genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*. Aquac. Int. 23, 1189–1201.

Li, Y., Bordinhon, A.M., Davis, D.A., Zhang, W., Zhu, X., 2013. Preotien:energy ratio in practical diets for Nile tilapia *Oreochromis niloticus*. Aquac. Int. 21, 1109–1119.

Li, J., Liu, G., Li, C., Deng, Y., Tadda, M.A., Lan, L., Zhu, S., Liu, D., 2018. Effects of different solid carbon sources on water quality, biofloc quality and gut microbiota of Nile tilapia (*Oreochromis niloticus*) larvae. Aquaculture 495, 919–931.

Liu, G., Ye, Z., Liu, D., Zhao, J., Sivaramasamy, E., Deng, Y., Zhu, S., 2018a. Influence of stocking density on growth, digestive enzyme activities, immune responses, antioxidant of *Oreochromis niloticus* fingerlings in biofloc systems. Fish Shellfish Immunol. 81, 416–422.

Liu, G., Zhu, S., Liu, D., Ye, Z., 2018b. Effect of the C/N ratio on inorganic nitrogen control and the growth and physiological parameters of tilapias [sic] fingerlings, *Oreochromis niloticu* [sic] reared in biofloc systems. Aquac. Res. 49, 2429–2439.

Liu, W., Luo, Z., Wen, H., 2018c. Effect of dietary protein levels and feeding rates on the growth and health status of juvenile genetically improved farmed tilapia (*Oreochromis niloticus*). Aquac. Int. 26, 153–167.

Liu, W., Luo, G., Chen, W., Tan, H., Wu, S., Zhang, N., Yu, Y., 2018d. Effect of no carbohydrate addition on water quality, growth performance and microbial community in water-reusing biofloc systems for tilapia production under high-density cultivation. Aquac. Res. 2446–2454.

Long, L., Yang, J., Li, Y., Guan, C., Wu, F., 2015. Effect of biofloc technology on growth, digestive enzyme activity, hematology, amd immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). Aquaculture 448, 135–141.

Lu, J., Furuya, Y., Yoshizaki, G., Sun, H., Endo, M., Haga, Y., Satoh, S., Takeuchi, T., 2009. Efficient productivity and lowered nitrogen and phosphorus discharge load from GH-transgenic tilapia (*Oreochromis niloticus*) under visual satiation feeding. Aquaculture 293, 241–247.

Luo, G.-Z., Avnimelech, Y., Pan, Y.-F., Tan, H.-X., 2013. Inorganic nitrogen dynamics in sequencing batch reactors using biofloc technology to treat aquaculture sludge. Aquac. Eng. 52, 73–79.

Luo, G., Zhang, N., Cai, S., Tan, H., Liu, Z., 2017. Nitrogen dynamics, bacterial community composition and biofloc quality in biofloc-based systems cultured *Oreochromis niloticus* with poly-β-hydroxybutyric and polycaprolactone as external carbohydrates. Aquaculture 479, 732–741.

Ma, F., Li, B., Li, X., Leng, X., 2015. Effects of extruded and pellets diets with differing protein levels on growth and nutrient retention of tilapia, *Oreochromis niloticus* × *O. aureus*. Aquac. Int. 23, 1341–1356.

Milstein, A., Peretz, Y., Harpaz, S., 2008. Culture of organic tilapia to market size in periphyton-based ponds with reduced feed inputs. Aquac. Res. 40, 55–59.

Noomhorm, A., Yamprayoon, J., 2000. Geosmin and off-flavor in Nile tilapia (*Oreochromis niloticus*). J. Aquat. Food Prod. Technol. 9, 29–41.

NRC (National Research Council), 2011. Nutrient requirements of fish and shrimp. In: National Research Council. The National Academies Press, Washington.

Perez-Fuentes, J.A., Hernandez-Vergara, M.P., Perez-Rostro, C.I., Fogel, I., 2016. C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. Aquaculture 452, 247–251.

Petersen, M.A., Hyldig, G., Strobel, B.W., Henriksen, N.H., Jørgensen, N.O.G., 2011. Chemical and sensory quantification of geosmin and 2-methylisoborneol in rainbow trout (*Oncorhynchus mykiss*) from recirculated aquacultures in relation to concentrations in basin water. J. Agric. Food Chem. 59, 12561–12568.

Podduturi, R., Petersen, M.A., Mahmud, S., Rahman, M.M., Jorgensen, N.O., 2017. Potential contribution of fish feed and phytoplankton to the content of volatile terpenes in cultured pangasius (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*). J. Agric. Food Chem. 65, 3730–3736.

Rawles, S.D., Green, B.W., Gaylord, T.G., Barrows, F.T., McEntire, M.E., Freeman, D.W., 2012. Response of sunshine bass (*Morone chrysops* × *M. saxatilis*) to digestible protein/ dietary lipid density and ration size at summer culture temperatures in the Southern United States. Aquaculture 356–357, 80–90.

Rawles, S.D., Green, B.W., McEntire, M.E., Gaylord, T.G., Barrows, F.T., 2018. Reducing dietary protein in pond production on hybrid striped bass (*Morone chrysops* × *M. saxatillis*): effects on fish performance and water quality dynamics. Aquaculture 490, 217–227.

Righetti, J.S., Furuya, W.M., Conejero, C.I., Graciano, T.S., Vidal, L.V.O., Michellato, M., 2011. Protein reduction in diets for Nile tilapia by amino acids supplementation based on the ideal protein concept. R. Bras. Zootece. 40, 469–476 (in Portuguese).

Satoh, H., Nakamura, Y., Ono, H., Okabe, S., 2003. Effect of oxygen concentration on nitrification and denitrification in single activated sludge flocs. Biotechnol. Bioeng. 83, 604–607.

Schrader, K.K., Blevins, W.T., 1993. Geosmin-producing species of *Streptomyces* and *Lyngbya* from aquaculture ponds. Can. J. Microbiol. 39, 834–840.

Schrader, K.K., Dennis, M.E., 2005. Cyanobacteria and earthy/musty compounds found in commercial catfish (*Ictalurus punctatus*) ponds in the Mississippi Delta and Mississippi-Alabama Blackland Prairie. Water Res. 39, 2807–2814.

Schrader, K.K., Green, B.W., Perschbacher, P.W., 2011. Development of phytoplankton communities and common off-flavors in a biofloc technology system used for the culture of channel catfish (*Ictalurus punctatus*). Aquac. Eng. 45, 118–126.

Schramm, A., Santegoeds, C.M., Nielsen, H.K., Ploug, H., Wagner, M., Pribyl, M., Wanner, J., Amann, R., de Beer, D., 1999. On the occurrence of anoxic microniches, denitrification, and sulfate reduction in aerated activated sludge. Appl. Environ. Microbiol. 65, 4189–4196.

Schroeder, G.L., 1978. Autotrophic and heterotrophic production of microorganisms in intensely-manured fish ponds, and related fish yields. Aquaculture 14, 303–325.

Shearer, K.D., 2000. Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. Aquac. Nutr. 6, 91–102.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd ed. W.H. Freeman and Company, New York.

Sweilum, M.A., Abdella, M.M., El-din, S.A.S., 2005. Effect of dietary protein-energy levels and fish initial sizes on growth rate, development and production of Nile tilapia, *Oreochromis niloticus* L. Aquac. Res. 36, 1414–1421.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Wastewater Engineering: Treatment and Reuse – Metcalf & Eddy, Inc. 4th ed. McGraw-Hill, New York.

Trung, D.V., Diu, N.T., Hao, N.T., Glencross, B., 2011. Development of a nutritional model to define the energy andprotein requirements of tilapia, *Oreochromis niloticus*. Aquaculture 320, 69–75.

Twibell, R.G., Brown, P.B., 1998. Optimal dietary protein concentration for hybrid tilapia *Oreochromis niloticus* × *O. aureus* fed all-plant diets. J. World Aquacult. Soc. 29, 9–16.

van den Berg, E.M., Boleij, M., Kuenen, J.G., Kleerebezem, R., van Loosdrecht, M.C.M., 2016. DNRA and denitrification coexist over a broad range of acetate/N-NO_3^- ratios, in a chemostat enrichment culture. Front. Microbiol. 7 (1842). https://doi.org/10.3389/fmicb.2016.01842.

van Rijn, J., Tal, Y., Schreier, H.J., 2006. Denitrification in recirculating systems: theory and applications. Aquac. Eng. 34, 364–376.

Wei, Y., Liao, S.-A., Wang, A.-L., 2016. The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. Aquaculture 465, 88–93.

Ye, J., Chen, J., Wang, K., 2016. Growth performance and body composition in response to dietary protein and lipid levels in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) subjected to normal and temporally restricted feeding regimes. J. Appl. Ichthyiol. 32, 332–338.

Zhao, D., Pan, L., Huang, F., Wang, C., Xu, W., 2016. Effects of different carbon sources on bioactive compound production of biofloc, immune response, antioxidant level, and growth performance of Litopenaeus vannamei in zero-water exchange culture tanks. J. World Aquacult. Soc. 47, 566–576.

Zimba, P.V., Grimm, C.C., 2003. A synoptic survey of musty/muddy odor metabolites and microcystin toxin occurrence and concentration in southeastern USA channel catfish (*Ictalurus punctatus* Ralfinesque) production ponds. Aquaculture 218, 81–87.