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ORIGINAL ARTICLE

Nitrogen retention, nutrient digestibility and growth efficiency of Nile tilapia (*Oreochromis niloticus*) fed dietary lysine and reared in fertilized ponds

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Abstract

Two diets were formulated, a low lysine (LowL) and a balanced lysine diet (BalL), containing 9.10 and 13.10 g lysine kg^{-1} feed, respectively. Twenty fish (30.2 ± 1.9 g) per tank were stocked in 110-L tanks, mounted in a recycling aquaculture system (RAS), and nine hundred fish (17.2 ± 1.6 g) per pond were stocked in 200 m^2 fertilized ponds (FPS). Four replicates in RAS and five replicates in FPS were assigned for each diet tested. Fish were fed with the experimental diets at a feeding rate based on metabolic body weight, twice daily for 70 days. Fish fed the BalL diet in RAS had a higher yield (kg m^{-3}), specific growth rate, nitrogen retention efficiency (%), protein efficiency ratio (g g^{-1} protein), protein content and essential amino acid content, as well as a better feed conversion ratio ($p < .05$). Lysine levels did not significantly affect fish survival (%), feed intake and apparent digestibility coefficients of nutrients. In contrast, in FPS, dietary lysine content did not ($p > .05$) affect the growth indices of nutrient utilization, survival (%), body composition and essential amino acids or nitrogen utilization efficiency. Percentage compositions of plankton in the gut contents and plankton abundances in water were approximately the same between diets. The Pearson correlation coefficient (r) between plankton abundance and growth in fish fed the LowL diet was .761 and −.961 for phytoplankton and zooplankton, respectively, compared with .50 and .54 in fish fed the BalL diet. The contribution of the natural food to nitrogen gain was 30% in fish fed the LowL diet, compared with 21% in fish fed the BalL diet ($p < .05$). The present study shows that natural food compensated for the deficiency of dietary lysine and improved the protein efficiency ratio by 46%, when compared to Nile tilapia grown in clear-water tanks.

KEYWORDS
dietary lysine concentration, natural food web, protein efficiency ratio, zero-water exchange

1 | INTRODUCTION

Food security of animal protein depends on responsible water use and sustainable aquaculture practices (Mansour et al., 2021). In conventional pond aquaculture systems, zero-water exchange helps to reduce water consumption (Sanchez et al., 2019). Nile tilapia (*Oreochromis niloticus*) is an important aquaculture species in Egypt and in many other countries across the world (Allam et al., 2020; Kord, Srour, et al., 2021). To make aquaculture less dependent on fishmeal, there is an ongoing shift towards replacement of fishmeal with plant protein ingredients (PPIs) in formulated aquafeeds (Staessen et al., 2019). Meanwhile, a common limitation of PPIs is low availability of the essential amino acid, lysine (Richter et al., 2021). The dietary lysine requirement (NRC, 2011) for Nile tilapia ranges from 13.0 to 14.4 g kg^{-1} of diet or 51.0–57.0 g kg^{-1} of crude protein (Richter et al., 2021). In ponds, plankton assemblages provide many valuable nutrients, including essential amino acids, nitrogen and phosphorous (Kabir et al., 2019; Kolmakova & Kolmakov, 2019). For example, nitrogen cycles through the food web in the pond between inorganic and organic forms, nourishing autotrophic and heterotrophic organisms with minimum loss in bioavailability (Nava & Leoni, 2020). The aim of the current research was to investigate the contribution of natural food to the lysine requirement for Nile tilapia, when reared in fertilized ponds with zero-water exchange.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Two separate experiments were conducted simultaneously for 70 days; in the first experiment, fish were raised in tanks within an indoor recirculating aquaculture system, (RAS) and in the second experiment, fish were raised in fertilized pond systems (FPS). In the FPS experiment, there were five replicated ponds per treatment, while, in the RAS experiment, there were four replicated tanks per treatment.

2.1.1 | Feed preparation

Two isonitrogenous (274.0 g kg^{-1} of diet) and isolipidic (67.7 g kg^{-1} of diet) diets were prepared, differing in lysine content. The first diet was formulated using ingredients low in lysine content to obtain a lysine deficient (LowL) diet. In the second diet, 5 g L-Lysine HCl 98% kg^{-1} was added, replacing 5 g kg^{-1} dried distillers grain (maize) in the ingredients, to obtain a diet that was not deficient in lysine, referred to as the lysine balanced (BalL) diet. The test diets were produced as floating extruded feed, at an extrusion temperature of 110–130°C, to obtain a moisture content after extrusion of 24%–27%. The feed pellets were subsequently dried, oil-coated, cooled and packed in 25 kg plastic bags by Skretting Egypt, Nutreco. The ingredients used and the macro-nutrient composition of essential amino acids (EAA) and non-essential amino acids (NEAA) in the extruded pellets were analysed according to AOAC (2016), using a Biochrom 30+ Amino Acid Analyzer and Ezchrom Elite software (Table 1). The BalL diet contained 13.10 g lysine kg^{-1} feed, and the LowL diet contained 9.10 g lysine kg^{-1} feed NRC (2011).

2.1.2 | Aquaculture system preparation

Eight settling column tanks, containing 110-L, were used in the recirculating aquaculture system (RAS). This facility was at the joint Research and Development unit, of the WorldFish Center and Skretting, Abbassa, Egypt. The hydraulic retention time in each tank was about 13 min. The RAS contained a submerged moving bed biofilter (1000 L), a drum filter for solid waste removal, a sump, a trickling filter and a UV-lamp.

Ten 200-m^2 (10 m × 20 m) fertilized ponds, with a water depth of 1 m each, were used. Each pond was fertilized with 2.5 g urea m^{-2} (46.5% N) and 2.5 g mono-superphosphate m^{-2} (15.5% P_2O_5), 3 weeks before fish stocking. On the short downwind side in each pond, three large frame airlifts aerated the ponds in the morning to avoid early morning oxygen depletion. No water was discharged during the experimental period (zero-water exchange). Ten per cent of water volume in the fertilized ponds was added every 15 days to compensate for evaporation and seepage loss.

Fingerlings of 9th generation genetically improved Abbassa Nile tilapia (GIANT), hatched at the WorldFish Centre's hatchery were used in the two experiments (Ibrahim et al., 2019). Twenty fish with an average weight of 30.2 ± 1.9 g and 900 fish with an average weight of 17.2 ± 1.6 g were stocked in each tank and pond, respectively. All fish used in the experiment were anaesthetized using clove oil (50 mg L^{-1}) before weighing and stocking. On the day of stocking, three samples of 10 and 50 fish randomly taken fish from the base populations stocked in the tanks and ponds, respectively, were taken for analysis of their initial proximate body composition. These fish were euthanized using an overdose of clove oil (450 mg L^{-1}) and deep-frozen at −20°C until analysis. Fish were hand-fed twice daily at rations of 24 g $kg^{-0.8}$ day^{-1} in RAS and 18 g $kg^{-0.8}$ day^{-1} in FPS, respectively.

2.2 | Monitoring and sampling

2.2.1 | Water quality

In the RAS, dissolved oxygen (DO), water temperature and pH were measured daily in the outlet water from the sump, using the NileBOT™ monitoring system (Conative Lab). Total ammonia nitrogen (TAN), nitrite-N (NO_2-N) and nitrate-N (NO_3-N) were measured weekly in the common outlet of the tanks, using water quality test kits (Visocolor ECO; Giatsis et al., 2014).

In each FPS, water temperature and DO were measured daily using an automatic probe (Hanna HI-9147). Total ammonia-N,

TABLE 1 Ingredients, nutrient composition and amino acids of lysine diets

Ingredient (g kg^{-1} DM)	Basal diet (BalL)	Test diet (LowL)
Wheat bran	200.0	
Dried distillers grain (maize)	150.0	155.0
Maize	140.0	
Wheat middlings	119.0	
Rice bran (14% CP)	100.0	
Sunflower meal (36% CP)	75.0	
Maize gluten meal (60% CP)	75.0	
Poultry meal	50.0	
Soybean meal (46% CP)	50.0	
Calcium carbonate	13.0	
Fish oil	10.0	
Monocalcium phosphate	4.0	
Skretting Premix PX STD[a]	3.0	
L-Threonine	2.0	
DL-Methionine	2.0	
L-Lysine HCl 98%	5.0	0.0
Sand (marker)	2.0	
Total	1000	1000
Analysed macro-nutrients (g kg^{-1} of diet)		
Dry matter (g kg^{-1} wet weight)	890.4	889.9
Crude protein (CP)	276.8	272.8
Crude fat (CF)	67.7	68.0
Crude fibre	59.8	60.0
Crude ash	75.2	77.2
Analysed essential amino acids (g kg^{-1} of diet)		
Methionine	5.20	5.00
Arginine	13.20	13.10
Lysine	13.10	9.10
Lysine (g kg^{-1} CP)	47.30	33.41
Threonine	10.41	9.41
Histidine	5.40	5.22
Isoleucine	9.21	8.61
Leucine	20.41	19.70
Phenylalanine	11.62	11.21
Valine	10.80	10.71
Analysed non-essential amino acids (g kg^{-1} of diet)		
Arginine	13.21	13.10
Aspartic acid	18.00	18.11
Tyrosine	8.11	7.71
Serine	10.10	8.60
Proline	15.32	15.50
Glycine	11.12	11.01
Glutamic acid	42.00	41.91
Alanine	12.32	12.11
Cystine	6.10	6.21

Note: Tryptophan and asparagine are not appearing in samples.

[a]Skretting standard vitamin and minerals premix exceeds levels for fish recommended by (NRC, 2011).

nitrite-N, nitrate-N and pH were measured at 8.00 AM two times per week. N-compounds were measured using a HACH test kit (model NI-8), following the Hach methodology Hach (1992).

Transparency was measured in each pond daily using the Secchi disc. Water samples of 1-L were collected at 30 cm depth to determine total suspended solids, total volatile solids and total alkalinity, using standard procedure 2540D, according to APHA (2017).

2.2.2 | Plankton identification and composition in ponds and fish gut

For phytoplankton investigation, 500 ml from four different points in each pond was collected at 30 cm depth, mixed with 1.5 ml of Lugol solution, and stored in the dark for 24 h, then siphoned to the volume of 100 ml. Thereafter, 1 ml was transferred to a Sedgewick-Rafter counting chamber (S-R cell). A phase-contrast microscope (Olympus) was used to determine the taxonomic status of phytoplankton at magnifications of ×100 to 400, using APHA (2005) methodology. Phytoplankton was identified up to phylum level, using Bellinger (1992) as a determination key.

For zooplankton investigation, 10 L of water was collected in each pond and filtered through a zooplankton net (mesh scale 50 μm), concentrated to 100 ml, preserved (on-site) in 5% formalin solution and counted in a Sedgewick-Rafter (S-R) cell under 100× magnification. Zooplankton were identified at phylum level using Edmondson et al. (1982) and Phan et al. (2015) as determination keys.

The phyto- and zooplankton concentrations in pond water were calculated as $n \times v \times 1000/V$.

Where "n" is the average number of plankton cells in 1 ml of water sample; "v" is the volume of the concentrated plankton after filtering (ml); and "V" is the volume of water before filtering (L).

At harvest, guts from 3 fish in each pond were collected by dissection and placed in 5% formalin. Then, 2.0 ml of distilled water was added to 1 ml of sample and this new solution was placed in a S-R cell for determining the plankton composition in the gut, according to

Abdel-Tawwab (2003). Besides plankton, the number of food items present in the gut was also counted. The percentage of plankton organisms in the gut contents was calculated as:

$$\text{Percentage plankton in gut content} = 100 \times \left(\frac{\text{number of plankton}}{\text{number of food items}}\right).$$

2.2.3 | Fish samples

At the end of the experiment, each RAS and FPS was emptied and all fish were harvested, counted and bulk weighed. Random samples of 50 fish per pond and 10 fish per tank were taken, euthanized with an overdose of clove oil (450 mg L^{-1}), weighed, and stored at −20°C for proximate body composition, as well as amino acid analysis by high-performance Amino Acid analyser (Biochrom 30), according to AOAC (2016).

2.3 | Calculated parameters

2.3.1 | Fish performance and feed utilization

The parameters of growth and feed utilization were calculated as follows:

$$\text{The total yield per RAS or FPS } \left(\text{kg m}^{-3}\right) = \frac{TW_{70} - TW_0}{V};$$

where TW_{70} was the total weight of the fish, TW_0 was the initial total weight of the fish and V was the water volume (m^3) of the systems;

$$\text{The survival } (\%) = \frac{Nb_{70}}{Nb_0} \times 100,$$

where Nb_{70} was the final number of fish and Nb_0 was the initial number of fish.

$$\text{The specific growth rate (SGR) (\% body weight/day)} = \frac{(\text{Ln}\,(W_{70}) - \text{Ln}\,(W_0))}{70} \times 100,$$

where W_{70} was the final weight and W_0 was the initial individual weight (g).

$$\text{Feed intake (FI) } \left(\text{g fish}^{-1}\right) = \frac{\text{Total feed consumed}}{(Nb_0/70)}.$$

$$\text{The feed conversion ratio (FCR) } \left(\text{g g}^{-1}\right) = \frac{FI}{(AWG)},$$

where FI is the feed intake and AWG is the average weight gain per fish ($W_{70} - W_0$, g $fish^{-1}$).

2.3.2 | Apparent digestibility coefficients in RAS

The digestibility of the nutrients and energy contained in the two experimental diets was determined after harvesting the fish from the growth experiment in the tanks. The BalL and LowL diets were ground and mixed with sand as acid-insoluble ash (AIA; 0.2% of total mass). Ten randomly selected fish were restocked in each tank and fed the same diet with AIA, at a ration of 16 g $kg^{-0.8}$ day^{-1}. Each day, prior to feeding, faeces from each tank were carefully collected. To avoid bacterial decay of the faeces, the collection tubes were immersed in ice, using the methodology of Maas et al. (2019). After collection, the faeces were frozen and combined into one batch per tank every 5 days, during a 15-day collection period and dried at 104 ± 1°C. For AIA analysis, samples of diets and dried faeces were incinerated at 600°C for 16 h, transferred to a 600 ml beaker, to which 100 ml of 4 M HCl was added, boiled for 5 min in a crude fibre digester (Labconco Corporation, Extraction Apparatus, G0002), filtered by Whatman No. 542, washed with 85°C distilled water (to reduce acidity) and finally returned to the crucible to be incinerated at 600°C for 16 h.

Acid-insoluble ash AIA (%) = $(w_F - w_E)/w_S \times 100$; where w_F is the weight of the crucible with ash of faeces (g), w_E is the weight of the empty crucible (g) and w_S is weight of faeces DM (g).

$$\text{The ADC } (\%) = \left(1 - \left(\left(\frac{AIA_D}{AIA_F}\right) \times \left(\frac{N_F}{N_D}\right)\right)\right) \times 100;$$

where AIA_D and AIA_F are % of acid-insoluble ash in diets and faeces, respectively, and N_F and N_D are the nutrient % in faeces and diet, respectively (Sales & Janssens, 2003).

2.3.3 | Nitrogen utilization

$$\text{The nitrogen intake } \left(N_i, \text{ mg N fish}^{-1}\text{ day}^{-1}\right) = FI \times N_{feed} \times 1000;$$

where N_{feed} is the fraction of nitrogen in the feed.

$$\text{The digestible nitrogen intake } \left(N_{digest}, \text{ mg N fish}^{-1}\text{ day}^{-1}\right) = \frac{N_i \times ADC_{nitrogen}}{100};$$

where $ADC_{nitrogen}$ is the apparent digestibility coefficient of nitrogen (%).

$$\text{Retained nitrogen } \left(RN, \text{ mg N fish}^{-1}\text{ day}^{-1}\right) = \frac{(N_{70} - N_0)}{70 \times 1000};$$

where N_{70} and N_0 are the nitrogen content in the fish at harvest and stocking (g), respectively. $N_{70} = W_{70} \times N_{fish70}$ and N_0 as $W_0 \times N_{fish0}$. N_{fish0} and N_{fish70} are the fractions of nitrogen in the fish on the days of stocking and harvesting, respectively.

$$\text{The nitrogen retention efficiency } \left(RN_{eff}, \%\right) = \frac{RN}{N_{digest}} \times 100;$$

$$\text{The protein efficiency ratio (PER)} = AWG/70/N_i \times 1000 \times 6.25;$$

Branchial and urinary loss (BUL, mg N $fish^{-1}$ day^{-1}) = N_{digest} − RN (Gonzalez et al., 2010).

2.3.4 | Nitrogen retention

All feed administered in two experiments were consumed by the fish. The protein growth in ponds based on natural food consumption was calculated according to Kabir et al. (2019):

$$\text{The retained nitrogen from feed per fish } \left(RN_{feed}, \text{ g fish}^{-1}\right) = \left(\frac{N_{digest} \times 70}{1000}\right) \times \left(\frac{RN_{eff}}{100}\right);$$

$$\text{The observed N - growth in ponds } \left(RN_{obs}, \text{ g fish}^{-1}\right) = \frac{RN \times 70}{1000};$$

$$\text{The contribution of the natural food in ponds } \left(RN_{nat\ food}, \text{ g fish}^{-1}\right) = RN_{obs} - RN_{feed};$$

$$\text{Contribution feed } \% = \frac{RN_{feed}}{RN_{obs}};$$

$$\text{Contribution natural food } \% = \frac{RN_{nat\ food}}{RN_{obs}}.$$

2.4 | Statistical analysis

The effect of lysine diets was analysed in two aquaculture systems as a separate experiment using one-way ANOVA. In FPS, all water quality parameters were calculated as weekly averages, and zooplankton and phytoplankton data were collected on three sampling dates. These observations in time were integrated in the analysis using repeated-measures ANOVA. Tukey test was used as a post hoc test to examine the differences between means at $p < .05$. Pearson correlation between plankton abundance and growth of Nile tilapia was also tested. All statistical analyses were done using SPSS statistical package (version 27 Inc.).

3 | RESULTS

3.1 | Water quality

In RAS, the water quality remained favourable for production during the culture period. The temperature was 25.3 ± 4.5°C; pH was 7.4 ± 2.4; dissolved oxygen was 7.7 ± 2.62 mg L^{-1}; TAN was 0.1 ± 0.01 mg L^{-1}; NO_2-N was 0.10 ± 0.04 mg L^{-1} and NO_3-N was 15 ± 2.0 mg L^{-1}. During the culture period, the water temperature and pH gradually declined, while NO_3-N increased. In FPS, the water quality parameters fluctuated between weeks ($p < .05$, Figure 1). The water temperature gradually declined during the period of the experiment. The NO_2-N levels increased, reaching 0.14 ± 0.04 mg L^{-1} at the end of the experiment. TAN ranged between 0.12 ± 0.04 and 0.18 ± 0.08 mg L^{-1}. Secchi disc transparency declined sharply from 35 to 14 cm at the end of week 1 and thereafter remained at 13.2 ± 1.2 cm until the end of the experiment. In contrast, transparency, TSS and TVS concentrations in the earthen ponds increased during the first 3–4 weeks of the experiment and thereafter remained within the range of 112–164 mg L^{-1} for TSS and 57–86 mg L^{-1} for TVS.

3.2 | Plankton abundance and percentage composition in gut contents

Overall, there were more phytoplankton phyla present than zooplankton phyla. There was no interaction ($p > .05$) between diet and time for any of the phytoplankton phyla (Table 2). Phytoplankton abundance was similar between diets but changed over time. Chlorophyta (Chlorophyceae) was the most abundant phytoplankton phylum, followed by Bacillariophyta (Bacillariophyceae); Cyanophyta (Cyanobacteria); Euglenophyta (Euglenophyceae); Cryptophyta (Cryptophyceae); and Dinophyta (Dinoflagellata). Additionally, the total phytoplankton abundance increased over time from 4.2 to 22.6 million L^{-1} for the BalL diet and from 5.7 to 35.2 million L^{-1} for the LowL diet. For zooplankton, Rotifera (*Brachionus plicatilis*) was the most abundant phylum, followed by Copepoda (Hexanauplia); Cladocera (Branchiopoda); and Protozoa. There was no interaction effect between treatment and time for all of the zooplankton phyla ($p > .05$), except for Rotifera ($p = .018$). Meanwhile, the LowL group affected the total zooplankton abundance by decreasing the abundance of Rotifera (*Brachionus plicatilis*) and Copepoda (*Hexanauplia*), which were found in increased numbers in the gut contents of the LowL fish (Table 2).

In ponds, the percentage of the phytoplankton and zooplankton phyla in the gut of Nile tilapia was similar for the LowL and BalL diets ($p > .05$; Table 3).

3.3 | Fish performance

In RAS, no fish mortality was observed. The BalL diet resulted in a significantly higher fish growth rate than the LowL diet ($p < .05$). Fish fed the BalL diet ate 16% more feed with an 18% lower FCR, compared with fish fed the LowL diet (Table 4). Similarly, fish fed the BalL diet had a higher SGR ($p \leq .01$) than fish fed the LowL diet.

In FPS, fish growth was not affected by lysine levels in diets ($p > .05$). The feed intake of both diets was similar ($p < .05$; Table 4).

3.4 | Apparent digestibility coefficients in RAS

The ADC values of both diets were not significantly different ($p > .05$; Table 5). Lipid was the most digestible nutrient (87%) followed by protein (82%), energy (75%), carbohydrate (63%), fibre (43%) and ash (30%).

3.5 | Fish proximate body composition and amino acid content

At the end of the experiment, dietary lysine levels did not affect the body composition, neither in RAS nor in FPS ($p > .05$), except for the protein % in fish raised in RAS, which was highest with the BalL diet ($p < .05$; Table 6).

In RAS, the BalL diet resulted in a significantly higher values in the fish of some EAAs (arginine, leucine, lysine, methionine,

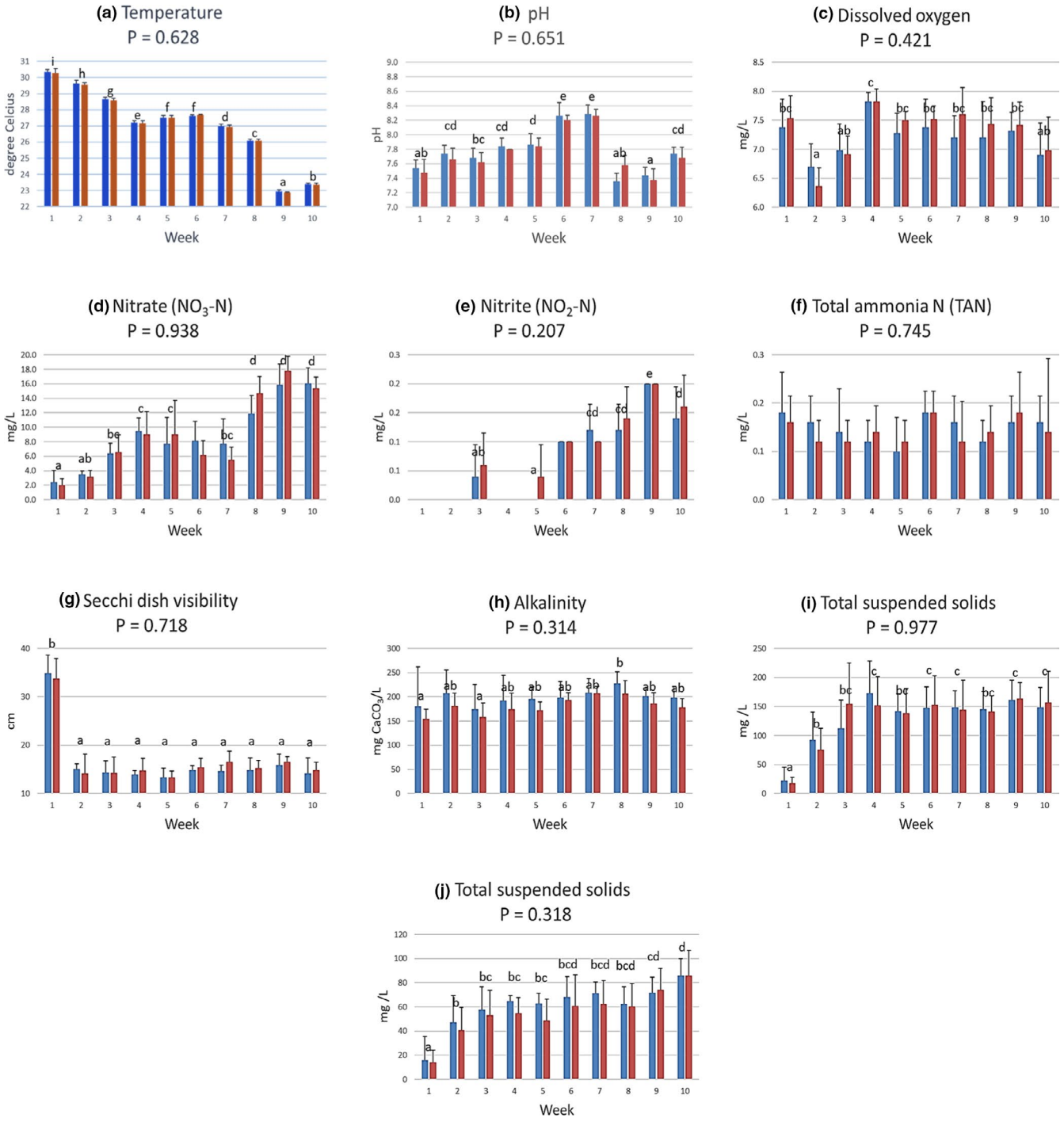

FIGURE 1 (a–j) Water quality parameters in fertilized ponds stocked with Nile tilapia (*O. niloticus*) fed a low lysine (LowL) or a balanced lysine (BalL) diet for 70 days. Data represented as means ± SE ($n = 5$). Blue is LowL diet, and red is BalL diet. Each line assigned with different letters is significantly different at $p < .05$

phenylalanine, threonine and valine) and all of the NEAA, when compared to the LowL diet ($p < .05$; Table 7).

In FPS, the values of EAAs arginine, histidine, leucine, lysine, threonine and valine were similar ($p > .05$) between the BalL and LowL diets. Furthermore, with the exception of glycine, cystine and tyrosine, the NEAAs values in the fish were higher ($p < .05$) with the BalL diet, when compared to the LowL diet (Table 7).

3.6 | Nitrogen utilization

The effect of dietary lysine levels on nitrogen utilization by Nile tilapia was different between tanks and ponds. In RAS, fish fed the LowL diet had a lower significant nitrogen intake (Ni) and nitrogen retention efficiency (RN_{eff}) than those fed the BalL diet (Table 8), resulting in a lower protein efficiency ratio (PER). In contrast, in FPS,

TABLE 2 Phytoplankton and zooplankton (organisms L^{-1}) in Nile tilapia (*O. niloticus*) ponds fed balanced (BalL) lysine and low (LowL) diets, sampled on days 23, 46 and 70 of the experiment for phytoplankton and days 50, 60 and 70 for zooplankton

FPS											
		Day 23		Day 46		Day 70			*p*-value		
	Phylum	BalL	LowL	BalL	LowL	BalL	LowL	SEM	Diet	Time	Diet × Time
Phytoplankton × 10^6	Chlorophyta	2.41	1.38	11.1	12.07	8.86	9.02	2.88	.975	.000	.740
	Cyanophyta	2.87	2.44	8.99	9.29	8.86	10.26	2.92	.694	.000	.782
	Euglenophyta	0.15	0.13	3.21	3.16	3.24	3.35	1.26	.979	.000	.989
	Bacillarophyta	0.30	0.13	6.70	9.94	9.02	11.72	5.40	.339	.001	.753
	Cryptophyta	0.01	0.08	0.10	0.76	1.03	0.81	0.45	.437	.000	.708
	Dinoflagellata	0.00	0.02	0.05	0.00	0.05	0.00	0.06	.194	.780	.274
	Total	5.73	4.18	31.05	35.21	22.61	35.15	9.32	.518	.000	.736
		Day 50		Day 60		Day 70					
Zooplankton × 10^3	Brachionus plicatilis	0.64	2.50	3.68	2.10	1.42	0.94	1.27	.887	.016	.018
	Cladocera	0.36	0.12	0.22	0.16	0.20	0.34	0.21	.500	.702	.159
	Copepoda	2.80	2.36	1.98	2.50	0.92	0.84	1.30	1.000	.018	.711
	Protozoa	0.02	0.00	0.02	0.00	0.00	0.02	0.03	.569	1.000	.282
	Total	3.74	4.88	5.79	4.69	2.49	2.11	2.33	.895	.028	.556

Note: The effects of diet, time and diet × time were tested by repeated-measures ANOVA with "diet" as between factor and "time" as within factor.

Abbreviation: SEM, Standard error of mean.

TABLE 3 Percentage plankton in gut contents of Nile tilapia (*O. niloticus)* fed a balanced lysine (BalL) diet or a low lysine (LowL) diet and reared in the fertilized ponds for 70 days

FPS				
Organisms	**BalL**	**LowL**	**SEM**	***p*-value**
Phytoplankton %				
Euglenophyceae	15.89	14.77	12.43	.926
Bacillariophyceae	36.06	39.03	10.17	.763
Cyanobacteria	26.56	23.59	10.49	.770
Chlorophyceae	21.49	22.61	11.74	.921
Zooplankton %				
Hexanauplia	18.89	26.03	17.80	.675
Brachionus plicatilis	42.22	72.38	24.39	.139
Branchiopoda	38.89	1.580	30.47	.145

Note: Data represented as means ± SEM ($n = 15$) corresponding means are significantly different at $p < .05$.

Ni, RN_{eff} and PER were not different between diets ($p > .05$), although there was a tendency ($p < .1$) for digestible nitrogen intake and retained nitrogen to be higher with the BalL diet, compared with the LowL diet (Table 8). Moreover, branchial and urinary loss was similar between diets in both experiments ($p > .05$).

3.7 | Contribution of natural food to growth

In FPS, fish fed the LowL diet realized 30% of their growth based on natural food intake. This was higher than for fish fed the BalL diet, which realized 21% of their growth based on natural food intake ($p < .05$; Figure 2). Moreover, the Pearson correlation coefficients (r) between plankton abundance and growth parameters indicate that plankton was more important for fish fed a low lysine diet (Table 9). Fish fed a LowL diet consumed more plankton to meet their requirements of lysine, resulting in a lower abundance of plankton in ponds where fish were fed the BalL diet. In ponds receiving the LowL diet, the correlation between phytoplankton abundance and growth was higher (0.761) compared with ponds receiving the BalL diet (0.504). For zooplankton, the correlation was negative, again with a larger value for the LowL diet (−0.961) than with the BalL diet (−0.540).

4 | DISCUSSION

4.1 | Water quality in aquatic systems

Regardless of diet, water quality during the experiments remained within pre-set limits for the culture of Nile tilapia, indicating that the water quality management of the culture systems was sufficient (Boyd & Tucker, 2012; Kord, Maulu, et al., 2021).

In RAS, the flow rate of water was 0.6–0.8 L min^{-1} per tank, to maintain good water quality throughout the experiment. In FPS, nitrification caused the NO_3-N concentration to increase slowly, but did not reach 20 mg L^{-1} by the end of the experiment, thereby causing no negative effect on fish performance. The water quality in ponds remained favourable to fish production, with the fish biomass staying within the carrying capacity of the ponds (Antony et al., 2006; Su et al., 2019).

4.2 | Fish performance and nitrogen utilization

In RAS, a restricted feed ration of 24 g $kg^{-0.8}$ day^{-1} was set for both diets; however, feed intake with the LowL diet was smaller than that with the BalL diet ($p = .029$). Dietary deficiency of lysine might lead to reduced feed intake and poor digestibility (Zhou et al., 2012). Low feed intake of the LowL diet in RAS resulted in poor protein utilization efficiency; the PER and RN_{eff} with the BalL diet were higher than with the LowL diet. With the LowL diet, fish compensated for the lysine deficiency by deamination of other amino acids in the liver, leading to increased nitrogen excretion relative to feed intake and reduced protein growth, compared with the BalL diet (Mozanzadeh et al., 2018). Michelato et al. (2016) mentioned that a dietary lysine concentration between 50 g kg^{-1} CP and 55.5 g kg^{-1} CP resulted in the best PER for market size Nile tilapia (2.7 g weight gain g^{-1} protein). This is similar to the PER obtained in our experiment with the BalL diet in RAS, but 46% lower than with both diets in ponds. Wang et al. (2020) reported that by increasing the amount of lysine up to 13.1 g kg^{-1} diet, weight gain, feed utilization, PER and nitrogen retention efficiency improved for yellow drum, *Nibea albiflora*. Ji et al. (2021) reported that by increasing the amount of lysine up to 1.84 g per 100 g diet, final body weight, weight gain, specific growth rate, feed conversion ratio and feed intake were improved for gibel carp *Carassius auratus gibelio*. The combined effect of reduced feed intake and protein utilization efficiency could explain the poor growth observed for Nile tilapia fed the LowL diet in RAS. In this regard, Prabu et al. (2020) showed that dietary lysine deficiency impaired the growth performance, body composition, nitrogen gain and lysine retention of Nile tilapia when fed a low lysine diet (14.3–17.5 g kg^{-1}). In the present study, the LowL diet resulted in less ($p < .05$) growth than the BalL diet. This finding agrees with previous studies, which reported that the growth performance, feed intake and feed conversion ratio of Nile tilapia were optimal at a dietary lysine level of 28–36 g kg^{-1} CP (Teodósio et al., 2020); digestible protein (Furuya et al., 2012) and protein retention were significantly higher in tilapia fed 30 g kg^{-1} CP (Teodósio et al., 2020). It was also previously observed that daily weight gain was better in Nile tilapia (27.5 g kg^{-1}) fed a diet containing 55.5 g lysine kg^{-1} CP, than in Nile tilapia fed a diet containing 43.9 g lysine kg^{-1} CP (Michelato et al., 2016).

At harvest, 0.60 and 0.65 kg Nile tilapia were produced per m^3 water volume in ponds fed the LowL and BalL diet, respectively. In contrast, in non-fertilizing ponds, a carrying capacity of 0.35 (kg m^{-3}) for a non-aerated pond can be maintained (Bosma & Verdegem, 2011). Additionally, the PER and RN_{eff} in FPS were higher than in RAS with the BalL and LowL diets. This positive improvement in

TABLE 4 Growth performance and nutrient utilization of Nile tilapia (*O. niloticus)* fed a balanced lysine (BalL) diet or a low lysine (LowL) diet and reared in aquaculture systems for 70 days

	RAS				FPS			
	BalL	LowL	SEM	*p*-value	BalL	LowL	SEM	*p*-value
Survival (%)	100.0	100.0	0.00	.00	97.58	97.64	1.89	.964
Total weight at stocking (TW_0, kg m^{-3})	6.11	5.99	0.44	.723	0.08	0.08	0.01	.750
Total weight at harvest (TW_{70}, kg m^{-3})	41.98[a]	31.42[b]	2.46	.002	0.65	0.60	0.03	.060
Yield (kg m^{-3})	35.86[a]	25.42[b]	2.13	.001	0.57	0.53	0.03	.091
Initial average weight (g $fish^{-1}$)	30.57	29.98	2.17	.735	17.38	17.06	1.70	.774
Final average weight (g $fish^{-1}$)	209.90[a]	157.10[b]	12.32	.002	146.20	136.51	7.78	.085
AWG (g $fish^{-1}$)	179.31[a]	127.11[b]	10.67	.010	128.80	119.51	8.56	.125
SGR (% body weight day^{-1})	2.75[a]	2.37[b]	0.07	.003	3.06	2.99	0.19	.581
Feed Intake (FI, g DM $fish^{-1}$ day^{-1})	3.38[a]	2.91[b]	0.18	.029	1.92	1.92	0.03	.970
FCR (g dry matter g^{-1})	1.32[a]	1.61[b]	0.03	<.001	1.04	1.12	0.06	.089

Note: Data represented as means ± SEM ($n = 5$) corresponding means are significantly different at $p < .05$.
Abbreviations: FCR, Feed conversion ratio; SGR, Specific growth rate.

TABLE 5 Apparent digestibility coefficients of nutrients in Nile tilapia (*O. niloticus)* fed with a balanced lysine (BalL) diet or a low lysine (LowL) diet and reared in a recycling aquaculture system (RAS) for 15 days

	RAS			
ADC (%)	BalL	LowL	SEM	*p*-value
Protein %	83.00	81.02	2.55	.300
Energy %	75.91	74.90	1.99	.494
Lipid %	87.50	85.62	4.30	.612
Carbohydrate %	64.12	62.20	3.65	.500
Ash %	35.20	25.91	9.77	.229
Crude fibre%	41.41	44.00	10.22	.736

Note: Data represented as means ± SEM ($n = 5$) corresponding means are significantly different at $p < .05$.

production (Wood et al., 2017). It is a conservation instrument for preserving ecological sustainability and equilibrium.

4.3 | Apparent digestibility coefficient, proximate body and amino acid content

In RAS, the ADC (%) of nutrients was similar for both diets ($p > .05$). Comparable ADCs of 87%, 87%, 77% and 29% for protein, lipid, energy and ash, respectively, were found by Tran et al. (2019), who fed Nile tilapia 25–35% CP. However, in the present study, poultry meal at 5% inclusion level was the only animal-based protein source in the diet. Several studies showed that plant-based ingredients generally have lower ADCs than animal-based ingredients (Al-Thobaiti et al., 2018; Cabral et al., 2013).

TABLE 6 Chemical composition (wet weight g kg^{-1}) of the whole body of Nile tilapia (*O. niloticus*) fed with a balanced lysine (BalL) diet or a low lysine (LowL) diet and reared in aquaculture systems for 70 days

	RAS					FPS				
	Day 0	Day 70				Day 0	Day 70			
Parameters	Initial	BalL	LowL	SEM	*p*-value	Initial	BalL	LowL	SEM	*p*-value
Dry matter	235.00 ± 0.30	303.01	314.02	1.91	.460	251.01 ± 0.00	313.00	311.01	2.25	.991
Protein	153.00 ± 0.31	160.02[a]	159.01[b]	0.01	.015	148.01 ± 0.11	159.01	159.01	0.98	.105
Lipid	60.10 ± 0.20	129.00	122.01	0.88	.338	64.02 ± 0.50	117.01	115.01	1.08	.989
Ash	34.01 ± 0.11	42.00	38.02	0.64	.401	43.11 ± 0.11	35.11	34.02	0.26	.701

Note: Data represented as means ± SEM ($n = 5$) corresponding means are significantly different at $p < .05$.

the PER and RN_{eff} found in pond treatments indicates that when a higher fraction of protein is fed, this results in fish biomass gain (Ovie & Eze, 2010). Furthermore, part of the nitrogen and energy excreted and defecated by the fish is partially reused by the plankton and other organisms in the food web, contributing in turn to fish

The body composition of tilapia fed the LowL and BalL diet in both systems falls within ranges reported for Nile tilapia by Hafedh (1999). Our results concur with Hua et al. (2019) who found that the body composition of Nile tilapia was significantly affected by the dietary lysine content, especially the protein % of body

TABLE 7 Amino acid profile (g kg^{-1} protein of dry weight) of Nile tilapia *O. niloticus* fed a balanced lysine (BalL) diet or a low lysine (LowL) diet and reared in the aquaculture systems for 70 days

	RAS				FPS			
	BalL	LowL	SEM	*p*-value	BalL	LowL	SEM	*p*-value
Essential amino acids								
Arginine	34.60[a]	33.72[b]	0.05	.02	41.00	40.61	0.03	.64
Histidine	11.21	11.10	0.02	.85	14.12	13.22	0.11	.39
Isoleucine	20.61	20.20	0.01	.23	26.00[a]	20.90[b]	0.31	.01
Leucine	32.91[a]	30.61[b]	0.05	.01	38.20	37.10	0.09	.14
Lysine	38.72[a]	37.10[b]	0.09	.01	41.71	40.71	0.02	.08
Methionine	12.20[a]	10.62[b]	0.03	.01	17.81[a]	12.91[b]	0.28	.02
Phenylalanine	23.11[a]	21.31[b]	0.04	.01	26.10[a]	23.02[b]	0.17	.02
Threonine	26.10[a]	23.41[b]	0.06	.02	26.62	27.21	0.07	.38
Valine	28.60[a]	27.60[b]	0.06	.04	36.70	35.42	0.03	.08
Non-essential amino acids								
Aspartic acid	57.51[a]	50.81[b]	0.15	.02	5.94[a]	57.11[b]	0.15	.03
Serine	23.21[a]	20.32[b]	0.06	.01	28.01[a]	24.9[2b]	0.17	.04
Glycine	52.40[a]	44.32[b]	0.18	.03	58.81	54.20	0.31	.06
Alanine	44.30[a]	33.81[b]	0.23	.01	48.50[a]	43.62[b]	0.28	.03
Cystine	6.00[a]	1.40[b]	0.10	.01	6.80	6.20	0.07	.35
Glutamic acid	81.72[a]	70.60[b]	0.25	.01	84.61[a]	81.51[b]	0.18	.01
Proline	33.52[a]	25.80[b]	0.17	.02	38.21[a]	34.51[b]	0.21	.02
Tyrosine	13.51[a]	11.31[b]	0.05	.03	17.50	15.01	0.17	.06

Note: Data represented as means ± SEM ($n = 5$) corresponding means are significantly different at $p < .05$.

TABLE 8 Nitrogen utilization of Nile tilapia (*O. niloticus*) fed with a balanced lysine (BalL) diet or a low lysine (LowL) diet and reared in aquaculture systems for 70 days

	RAS				FPS			
	BalL	LowL	SEM	*p*-value	BalL	LowL	SEM	*p*-value
Nitrogen intake (N_i; mg $fish^{-1}$ day^{-1})	150.01[a]	127.00	9.11	.022	84.0	83.21	1.69	.475
Digestible nitrogen intake (mg $fish^{-1}$ day^{-1})	125.10[a]	106.01[b]	7.33	.018	71.0	69.01	1.41	.056
Retained nitrogen (mg $fish^{-1}$ day^{-1})	65.70[a]	46.80[b]	3.83	<.001	47.41	43.60	3.13	.091
Nitrogen retention efficiency (RN_{eff}; %)	52.71[a]	44.00[b]	0.97	<.001	67.01	63.40	4.29	.221
Protein efficiency ratio (PER; g g^{-1} protein)	2.70[a]	2.20[b]	0.97	<.001	3.90	3.70	0.24	.168
Branchial and urinary loss (mg $fish^{-1}$ day^{-1})	59.60	59.00	3.54	.815	23.41	25.40	2.92	.311

Note: Data represented as means ± SEM ($n = 5$) corresponding means are significantly different at $p < .05$.

composition. Meanwhile, Michelato et al. (2016) did not find any effect on final body composition of Nile tilapia fed graded levels of lysine. In addition, in FPS, most of the EAA content was similar between the BalL and LowL diets. This finding agrees with previous studies that reported that amino acid catabolism increased in Nile tilapia fed the 36.0 g kg^{-1} CP diet (Teodósio et al., 2020). This means that there is another source of protein in FPS, which enhanced amino acid retention in the muscles. Microalgae and zooplankton are the rich sources of high-quality protein, with lysine providing 7.1% and 8.6% of the EAA pool in the organisms (Kolmakova & Kolmakov, 2019), thus contributing to Nile tilapia production in ponds.

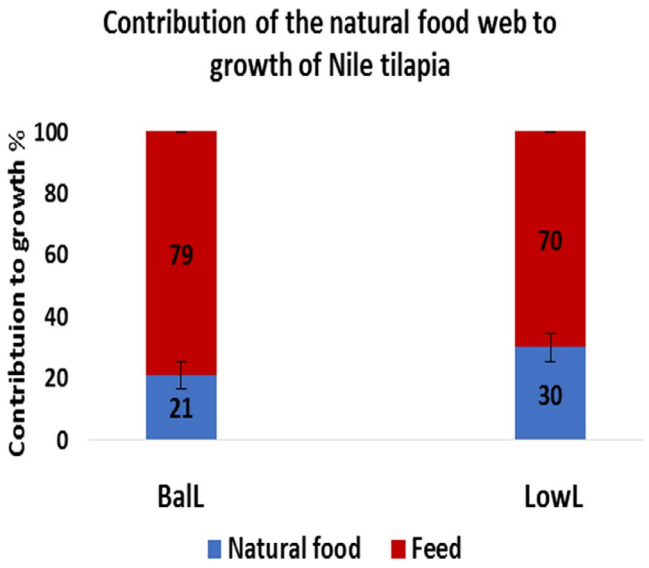

FIGURE 2 The contribution (%) of natural food to nitrogen retention of Nile tilapia, when fed a balanced lysine (BalL) diet or a low lysine (LowL) diet and reared in a fertilized pond system for 70 days

TABLE 9 Pearson correlation (*r*) between plankton abundance and Nile tilapia (*O. niloticus*) growth, when fed a balanced lysine (BalL) diet or a low lysine (LowL) diet and reared in fertilized ponds for 70 days

FPS		
	Pearson correlation (*r*)	*p*-value
BalL		
Phytoplankton versus growth	.504	.664
Zooplankton versus growth	−.540	.637
LowL		
Phytoplankton versus growth	.761	.449
Zooplankton versus growth	−.961	.179

Note: p-value represents 2-tailed significance.

4.4 | Natural food compensated low dietary lysine

In contrast to RAS, feeding the low lysine diet in ponds did not affect growth ($p > .05$) across all fish performance indices. This suggests that the fish obtained lysine from a source other than the feed, allowing the fish to balance their requirement for lysine (Furuya & Furuya, 2010). Our results, in percentage composition of plankton in gut contents of Nile tilapia reared in FPS and fed LowL and BalL diets, were similar to results previously published by (Abdel-Tawwab, 2011; Vasconcelos et al., 2018), who reported that Nile tilapia is an omnivorous filter-feeding fish, which can efficiently utilize phytoplankton and zooplankton resources. The use of floating pellets in the present study allowed us to check feed intake in FPS. This, in combination with the lower FCR realized in FPS compared with RAS, suggests that natural food contributed to total pond production. In ponds receiving the LowL diet, natural food intake contributed 30% to total pond production, which was higher than the 21% contribution in ponds fed the BalL diet ($p < .05$). Other studies reported contributions of natural food to total pond production of up to 60% (Kabir et al., 2019; Pucher & Focken, 2017). In this study, the fish biomass reached 0.6 and 0.65 kg m^{-3} in ponds fed the LowL and BalL diet, respectively. Considering the ponds had zero-water exchange, a possible explanation might be that our ponds operated close to carrying capacity when harvested. When approaching the maximum biomass and feed load, the contribution of natural food to pond production will quickly diminish (Hermsen et al., 2020). Further research to test this hypothesis is recommended.

Overall, the contribution of natural food to growth, nutrient utilization and nitrogen retention efficiency was higher in ponds fed the LowL diet. This result is supported by the positive and negative correlations between fish growth and phytoplankton and zooplankton abundances, respectively. Filter-feeding Nile tilapia may indirectly support phytoplankton production by grazing on zooplankton and reducing its abundance (Leoni et al., 2018; Vasconcelos et al., 2018). Ovie and Ovie (2006) and Rasdi et al. (2020) reported that the lysine content of laboratory-cultured Rotifera and Cladocera (Branchiopoda) could range between 86 and 107 g kg^{-1} CP, making lysine their most abundant essential amino acid. This possible high content of lysine in zooplankton in our experiment might in part explain why the correlation was stronger for the LowL diet than for the BalL diet.

5 | CONCLUSION

The present study shows that natural food in fertilized ponds compensates for the deficiency of dietary lysine in a plant-based diet, improving both nitrogen retention and the protein efficiency ratio, which was up to 46% higher than in Nile tilapia grown in clear-water tanks.

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CONFLICT OF INTEREST

The authors haven't conflict of interest.

DATA AVAILABILITY STATEMENT

Raw data were generated at Wageningen University and WorldFish Center. The data that support the findings of this research are available from the corresponding author upon request.

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