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# Metabolic growth, plankton selectivity, haemato-biochemical and intestinal morphometry of Nile tilapia (*Oreochromis niloticus*) fed a lysine-deficient diet in earthen ponds

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ABSTRACT

Sustainable aquaculture requires the efficient use of natural pond resources as well as supplements to meet the dietary requirements of fish species. However, aquaculture diets are often produced without considering the lysine content of the natural production in the pond. As such, two plant-based diets, a control diet (L-Con) supplemented with 5.00 g lysine $kg^{-1}$ diet and a lysine deficient diet (L-Def) without supplementation were fed to Nile tilapia raised in ponds with five replicates ponds for each diet. The aim was to investigate the effect of L-Def diet on plankton selectivity, metabolic growth, haemato-biochemical parameters, gut content and intestinal morphometry. Fish were fed a fixed ration of 18 g $kg^{-0.8}$ $d^{-1}$ for 10 weeks. During the experimental period, plankton species in the fish guts were identified and categorised to phylum level: phytoplankton phyla – Euglenophyta, Bacillariophyta, Cyanobacteria, and Chlorophyta; zooplankton phyla - Copepoda, Rotifera, and Cladocera. Fish in ponds receiving the L-Def diet selected positively for all phyto and zooplankton phyla but the selectivity was only significantly higher for Bacillariophyta with fish in ponds receiving the L-Con diet ($P < 0.05$). The numerical index (N%) of plankton in the gut content of Nile tilapia was higher in the L-Def treatment (48%) than in the L-Con treatment (38%). Fish growth and mortality were not significantly different between the two dietary treatments. The organo-somatic indices and haemato-biochemical parameters were similar between diets. The fish intestinal cross-sections from the L-Con and L-Def treatments showed numerous goblet cells in the proximal, middle, and distal gut, while the distal gut showed a partial serosal surface. Meanwhile, intestine morphometry sections of the distal villi length, submucosal layer, muscular layer and mid villi width, proximal / mid goblet cell counts and proximal lamina propria, were significantly higher in the L-Def treatment than in the L-Con treatment. The adverse impacts of dietary lysine deficiency on growth and fish health were reduced through plankton selectivity by Nile tilapia reared in ponds.

## 1. Introduction

The strategies for producing better aqua-diets, enabling improved fish health and environmental sustainability should be considered (Hoseinifar et al., 2016; Kord et al., 2021a). Furthermore, as the aquaculture industry expands, dietary protein sources have shifted, with less dependence on fish meal and more reliance on plant protein ingredients (Allam et al., 2020; Maulu et al., 2021). This shift, while maintaining growth, comes with its own set of difficulties and complicated interactions with the health of the fish, in terms of an imbalance in

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essential amino acids (Jobling, 2016), which are one of the most essential nutrients for fish health, since they are involved in the synthesis of immune cells and tissues (Li et al., 2009; Trichet, 2010). A lysine deficient diet leads to less feed intake, reduced growth, and high mortality (Huang et al., 2021a; Khalil et al., 2021). Interestingly, aqua-diets are usually produced without considering the possible contribution of the production system to the lysine requirements of the cultured fish species. In a clear-water system, the dietary lysine required for *Oreochromis niloticus* is around 13.0–14.4 g $kg^{-1}$ (NRC, 2011). In the recycling aquaculture system (RAS), fish fed the dietary lysine requirement had a higher nitrogen retention efficiency, growth performance, feed utilisation, and proximate composition than the lysine deficient diet (Khalil et al., 2021). Khalil et al. (2021) reported that dietary lysine may be complemented through the natural food abundant in the ponds, reducing the need to add synthetic lysine to the diet and increasing the nitrogen retention as well as protein efficiency ratio. In ponds, the latter was up to 46% higher than in clear-water tanks. For instance, typical tropical ponds are rich in rotifers and copepods, which have been identified as healthy sources of lysine (Ovie, Ovie, 2006; Rasdi et al., 2020). This is supported by positive and negative correlations among fish performance, plankton abundance and plankton consumption by Nile tilapia. This consumption was more pronounced in ponds fed the L-Def diet than in ponds fed the L-Con diet (Khalil et al., 2021). In silvery black porgy, lysine deficiency may result in a decrease in total immunoglobulin (Yaghoubi et al., 2017; Mozanzadeh et al., 2018). According to Lall (2000), proteo-energetic malnutrition causes significant nutritional stress and affects health in most farmed fish when a nutrient-deficient diet is fed which affect haematological parameters and serum biochemical indices in a (Trichet, 2010). Previous research has shown that dietary lysine affected the intestinal structure and the transcription of amino acids, which facilitated the intestinal absorption and synthesis of several amino acids (He et al., 2013, 2016). Furthermore, lysine is important for the growth of the gastrointestinal tract and conducive to fish health (Furuya, Furuya, 2010; Hamid et al., 2016). Additionally, lysine is among the basic components for the synthesis of proteins, peptides, and non-peptide compounds in fish, and it plays a crucial role in physiological and biochemical functions (Liao et al., 2015). In 2001, a selective breeding programme was launched in Abbassa, Egypt, with the aim of developing and producing a genetically improved Nile tilapia, known as the "Genetically Improved Abbassa Nile Tilapia (GIANT)" strain (Ibrahim et al., 2019). This strain has high growth performance and survival rates from seed to harvest size, making it an economically sustainable choice (Ibrahim et al., 2013). Therefore, the current research was done to investigate the effect of a lysine deficient diet on plankton selectivity, metabolic growth, haemato-biochemical parameters, gut content and intestinal morphometry of Nile tilapia (*Oreochromis niloticus*) raised in fertilised earthen ponds.

## 2. Materials and methods

### 2.1. Ethical Statement

The current study was performed following relevant institutional and international guidelines. Ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC), Alexandria University (AU/ 2019/040356).

### 2.2. Fish rearing conditions

The experiment was performed at the WorldFish center, Abbassa, Egypt. The experimental pond system consisted of 10 units of 200-$m^2$ active suspension (AS) ponds (10 m x 20 m x 1 m); each pond having three large airlifts. Before stocking, each pond received 1 kg / pond of superphosphate (15.5% $P_2O_5$) and 1 kg / pond of urea (46.5% N).

A total of 9000 mono-sex 9th generation genetically improved Abbassa Nile tilapia (*O. niloticus*) were obtained from the WorldFish Hatchery, Abbassa, Egypt. Each pond was stocked with 900 fish (17.2 ± 1.6 g). During culture there was no water exchange, and water was added to each pond every 15 days to compensate for water loss due to evaporation and seepage. The two experimental diets were fed at a ration of 18 g $kg^{-0.8}$ $d^{-1}$ of metabolic body weight, twice daily, for ten weeks.

### 2.3. Feed ingredients and diet preparation

Two isonitrogenous (272.8 g $kg^{-1}$) and isolipidic (67.7 g $kg^{-1}$) diets were formulated. The control diet (L-Con) was supplemented by 5 g of synthetic L-lysine 98.5%, while the lysine deficient diet (L-Def) had no synthetic lysine added (Table 1). The experimental diets were prepared by Skretting Egypt, Nutreco Company. All the ingredients were mixed, then underwent the extrusion process of high pressure (34–37 atmospheres), moisture (24–27%) and high temperature (123–150 °C for 2 min). The resulting extruded pellets (3-mm diameter, 2-mm length) were then vacuum coated (preheating the fat to 60 °C) and cooled before being packed in plastic bags (25 Kg). The proximate chemical composition and total amino acids of the experimental diets were analysed according to AOAC (2016) by Near-infrared spectroscopy (NIRS) and

**Table 1**
Ingredient and nutrient composition g $kg^{-1}$ wet weight of the lysine diets.

| Ingredient | L-Con | L-Def |
|---|---|---|
| Soybean Meal (46% CP) | 50.0 | 50.0 |
| Maize Gluten meal (60% CP) | 75.0 | 75.0 |
| Maize | 140.0 | 140.0 |
| Wheat bran | 200.0 | 200.0 |
| Sunflower Meal (36% CP) | 75.0 | 75.0 |
| Rice bran | 100.0 | 100.0 |
| Wheat middlings | 119.0 | 119.0 |
| Maize Distillers Dried Grains (27% CP) | 150.0 | 155.0 |
| Poultry meal (64% CP) | 50.0 | 50.0 |
| Fish oil | 10.0 | 10.0 |
| L-Lysine HCl 98.5%[a] | 5.0 | 0.0 |
| L-Threonine min 98% | 2.0 | 2.0 |
| DL-Methionine 99% | 2.0 | 2.0 |
| Mono-calcium phosphate | 4.0 | 4.0 |
| Skretting standard premix®[b] | 3.0 | 3.0 |
| Calcium carbonate | 15.0 | 15.0 |
| Total | 1000 | 1000 |
| **Proximate Composition g $kg^{-1}$** | | |
| Dry matter | 890.4 | 889.9 |
| Crude protein | 276.8 | 272.8 |
| Crude fat | 67.7 | 68.0 |
| Crude fibre | 59.8 | 60.0 |
| Ash | 75.2 | 77.2 |
| **Essential Amino Acid (g $kg^{-1}$)** | | |
| Lysine | 13.10 | 9.10 |
| Threonine | 10.41 | 10.50 |
| Methionine | 5.20 | 5.02 |
| Arginine | 13.20 | 13.10 |
| Histidine | 5.40 | 5.22 |
| Isoleucine | 9.21 | 9.61 |
| Leucine | 20.41 | 19.70 |
| Phenylalanine | 11.62 | 11.21 |
| Valine | 10.80 | 10.71 |
| **Non-essential Amino Acid (g $kg^{-1}$)** | | |
| Aspartic acid | 18.00 | 18.11 |
| Serine | 10.10 | 10.60 |
| Glycine | 11.12 | 11.01 |
| Alanine | 12.31 | 12.10 |
| Cystine | 6.11 | 6.20 |
| Glutamic acid | 42.00 | 41.91 |
| Proline | 15.32 | 15.50 |
| Tyrosine | 8.11 | 8.71 |
| Arginine | 13.21 | 13.10 |

[a] L-Lysine Monohydrochloride HCL—98.5%, Ajinomoto Heartland.
[b] Vitamin & mineral premix from Skretting® Company exceeds levels recommended by (NRC, 2011).

amino acid analyser (Biochrom 30) (Table 1).

### 2.4. Sampling collection

#### 2.4.1. Water quality measurements

Before the start of the experiment, one composite water sample was collected from ponds to measure initial water quality parameters. Temperature (ºC), pH and dissolved oxygen (mg $L^{-1}$) were measured twice daily by using an automatic probe (HI-9147, Hanna® instruments Inc., USA). Water samples of 1-L were collected from four different locations in each pond and mixed together and used to measure average total suspended solids (TSS) and total volatile solids (TVS) by taking 100-mL mixed with a vortex machine and passing it through Whatman glass fibre filter (GF/C pore size 1.2 μm) and thereafter dried and ashed to measure TSS and TVS weekly, following the 2540D procedure according to APHA (2017). Total ammonia-nitrogen (TAN) was measured using HACH test kit no. 2559833 (Hach, 1992).

#### 2.4.2. Plankton abundance

Plankton samples from fish ponds were measured weekly, 0.5 L water samples, taken from different locations in each pond were pooled into one sample per pond, to which 7.5 mL of Lugol solvent was added. The sample was stored in the dark for one day to allow the plankton to settle on the bottom and diluted to 100 mL. Phytoplankton concentration ($L^{-1}$) in the pond water was estimated according to APHA (2005). Phytoplankton was identified to phylum level, according to Bellinger (1992). Furthermore, 10 liter of water from each pond was collected, filtered through a zooplankton net, condensed to 100 mL and stored in a 5% formaldehyde solution. The zooplankton ($L^{-1}$) was then counted in a Sedgwick-Rafter chamber at 100 x magnification and identified to phylum level, according to Edmondson et al. (1982).

The plankton concentrations in water were calculated as

$$N\ (\text{organism}\ L^{-1}) = \frac{No\ of\ organisms\ in\ 1\ cm^3\ of\ sample\ X\ Conc.\ volume\ of\ sample\ cm^3}{Volume\ of\ water\ before\ filtering\ (L).}$$

#### 2.4.3. Gut contents indices

Three fish from each pond were collected randomly at the end of the experiment, the fish gut was dissected and the contents were removed and put into a bottle containing 5% formalin. In the laboratory, gut fullness was determined using the method defined by Cornelissen et al. (2015). Of each sample, 1 mL was placed in a Sedgwick-Rafter chamber for identification and concentration of plankton species in the gut according to (Abdel-Tawwab, 2003; Khalil et al., 2021). A phase-contrast microscope (Olympus, Japan) was used, at a magnification of x 100–400, to obtain plankton selectivity indices (Ivlev index) and selectivity ratio (Ivlev's forage ratio) according to the following formulas:

Plankton selectivity index (Ei) $= (W_i - n^{-1})\ (W_i + n^{-1})^{-1}$

Where, n is the phyto- and zooplankton count available; $W_i = r_i\ p_i^{-1} \sum (r_i\ p_i^{-1})^{-1}$

where $r_i$ is the phyto- and zooplankton % in the fish gut and $_{pi}$ the phyto- and zooplankton % pond water.

Plankton selectivity ratio (Ivlev's forage ratio) ($Ei^r$) $= r_i$ / $_{pi}$ (Ivlev, 1975; Tófoli et al., 2013).

Also, plankton, artificial feed, sand, plant parts and unidentified food items in the gut were counted to obtain their numerical indices (N%) (Abidemi-Iromini, 2019), according to the following formula:

$$\text{Numerical}\ indices\ (N\%) = \frac{\text{Total number particular food}\ item}{\text{Total number of food}\ items} \times 100$$

#### 2.4.4. Blood and tissue sampling

On harvest day, a random sample of 10 fish were collected from each pond and anesthetised by using clove oil (5 mL $L^{-1}$) before blood collection (Mansour et al., 2017). Blood samples were collected from the caudal vein of anaesthetised fish. Each blood sample was divided into two parts; one part for haematology and the other for biochemical assays. Haematological samples were stored as whole blood in heparinized tubes, while biochemical assays samples were centrifuged (1075 x g, 10 min, 4ºC) to obtain serum, which was stored at − 80ºC until analysis.

Afterwards, fish were euthanised with an overdose of clove oil (450 mL $L^{-1}$) (Mansour et al., 2017), the fish gut was then dissected for gut content analysis. The fillet, total viscera, and liver were weighed to calculate the somatic indices. The intestine was preserved in 10% formalin solution before histological analysis.

### 2.5. Analytical procedures and calculations

#### 2.5.1. Fish growth

Growth performance was determined using pooled data from all the fish per experimental unit in terms of final body weight (FBW), fish yield (FY), growth rate per metabolic body weight ($GR_{mbw}$), and thermal growth coefficient (TGC), which were calculated according to the following formulae:

Average weight gain (g) $= W_D - W_0$

Fish net yield (kg $ha^{-1}$) $= (W_D - W_0) \times S_D \times S_R \times A$ (Billah et al·, 2020)·

$GR_{mbw}$ (g $kg^{-0.8}$ $day^{-1}$) $= (W_D - W_0)$/ (MBW x D) (Terpstra, 2015)·

$TGC = (W_D^{1/3} - W_0^{1/3} / \sum_{i=1}^{D} Ti) \times 1000$ (Mansour et al·, 2017)·

where: $W_D$ is the final weight (g); $W_0$ is the initial weight (g); $S_D$ is the stocking density (fish $m^{-1}$), $S_R$ is the survival rate (%), A is the pond area ($m^2$), MBW is the metabolic body weight ($kg^{-0.8}$); D is the number of days of the experiment; $T_i$ is the mean daily temperature ($^0$C).

#### 2.5.2. Organo-somatic indices

For each fish sample, the condition factor and organo-somatic indices were calculated according to Khalil et al. (2019), as follows:

Condition factor $= 100$ [fish weight (g)/total length $(cm)^3$]·

Viscera-somatic index (VSI) (%) $= 100$ [viscera weight (g)/body weight (g)];

Hepato-somatic index (HSI) (%) $= 100$ [liver weight (g)/body weight (g)];

Gonadal-somatic index (GSI) (%) $= 100$ [Gonad weight (g)/body weight (g)]·

Fillet yield (%) $= 100$ [fillet weight (g)/body weight (g)]·

#### 2.5.3. Haematological parameters

Haemoglobin concentration (Hb), haematocrit value (Hct), red blood cell (RBC) count, and white blood cell (WBC) count in heparinized whole blood samples were analysed using an automated technical analyser (Celltac & MEK – 6400 JLK), according to Van, Zijlstra (1983). Mean Cell Volume (MCV), and Mean Cell haemoglobin (MCH), were

estimated based on Lewis (2006). White blood cell differential counts were calculated using the method according to Blumenreich (1990) and Mansour et al. (2017).

### 2.5.4. Serum biochemical assays

Total protein (g $dL^{-1}$), albumin (g $dL^{-1}$), triglycerides (g $dL^{-1}$), calcium (mg $dL^{-1}$), and phosphorus (mg $dL^{-1}$) levels in serum samples were determined by colorimetric assays using an assay kit supplied by Diamond Diagnostics, Cairo, Egypt, according to the parameter tested (Gornall et al., 1949; Doumas et al., 1971; Fossati, Prencipe, 1982). Activity of alkaline phosphatase (ALP, $UL^{-1}$) was determined according to Shun et al. (1994). Aspartate aminotransferase (AST, $UL^{-1}$), and alanine aminotransferase (ALT, $UL^{-1}$) were determined using commercial diagnostic kits (bioMérieux, France) according to Reitman, Frankel (1957).

### 2.5.5. Intestinal morphology

The fixed intestine samples were processed through the conventional paraffin embedding technique. Paraffin blocks were sectioned into 5 μm thick strips and subjected to hematoxylin and eosin (H&E) staining (light microscopy). In each sample, the length of muscular layer (mL), submucosal layer (SML), lamina propria (LP), villi length (VL) and villi width (VW) were measured in the proximal, middle and distal sections of the intestine, under an ocular microscope with a ruler (to the nearest 0.1 mm). For each parameter, five measurements were made from each of the three sections per sample and mean values were calculated (Wang et al., 2017; Khalil et al., 2019). Similarly, goblet cells (GC) in each of the sections were quantified by counting their number in each section to determine the mean abundance (Kord et al., 2021a).

### 2.6. Statistical analysis

All the measured parameters were analysed for both significant difference between both groups using one-way ANOVA. Duncan's multiple range test was used as a *post-hoc* test to compare between means at the $P > 0.05$ level. Statistical analysis was done using the SPSS statistical package (version 27 Inc. Chicago, Illinois).

## 3. Results

### 3.1. Water quality parameters

There were no significant ($P > 0.05$) effects of the tested diets on water quality: water temperature (27.01 ± 2.30 C°); pH (7.74 ± 0.31);

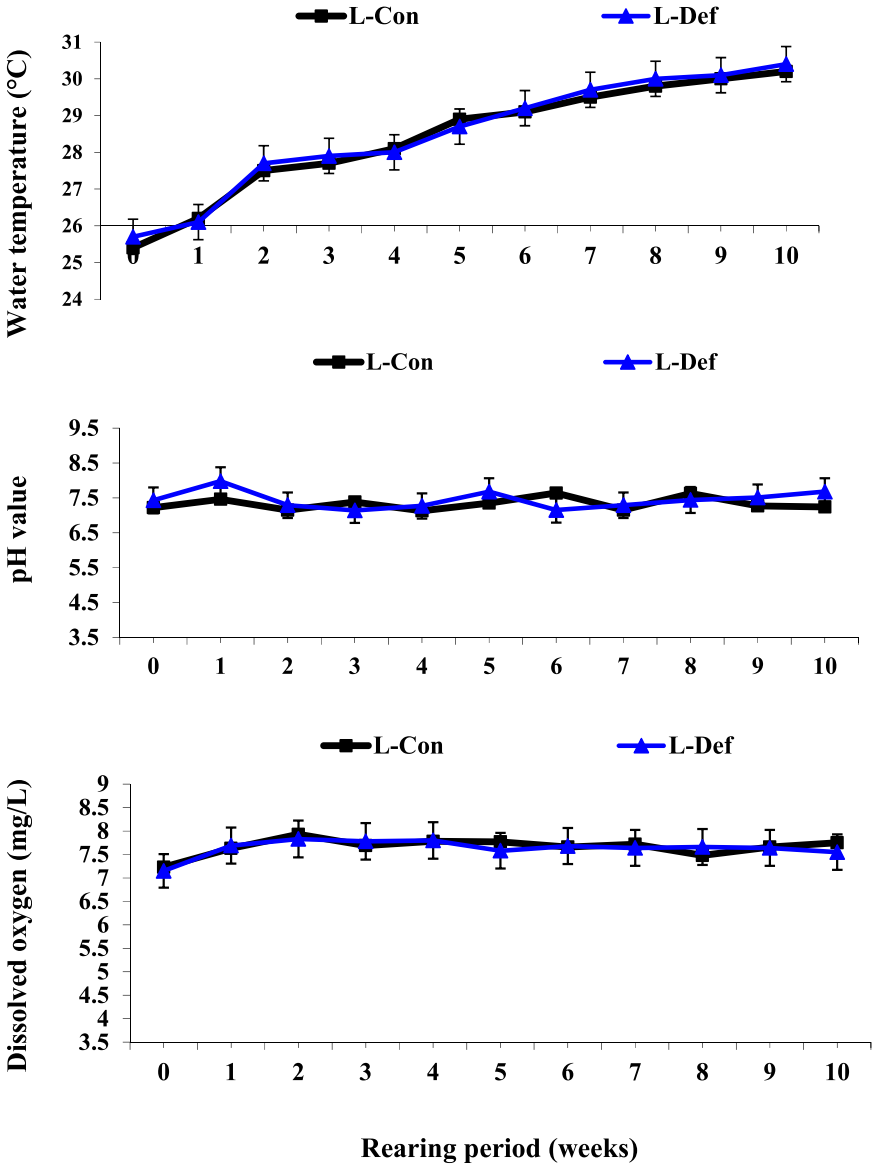


**Fig. 1.** Water temperature, pH, and dissolved oxygen values in ponds of Nile tilapia (*O. niloticus*) fed the lysine diets for 10 weeks, n = 5; means ± SE.

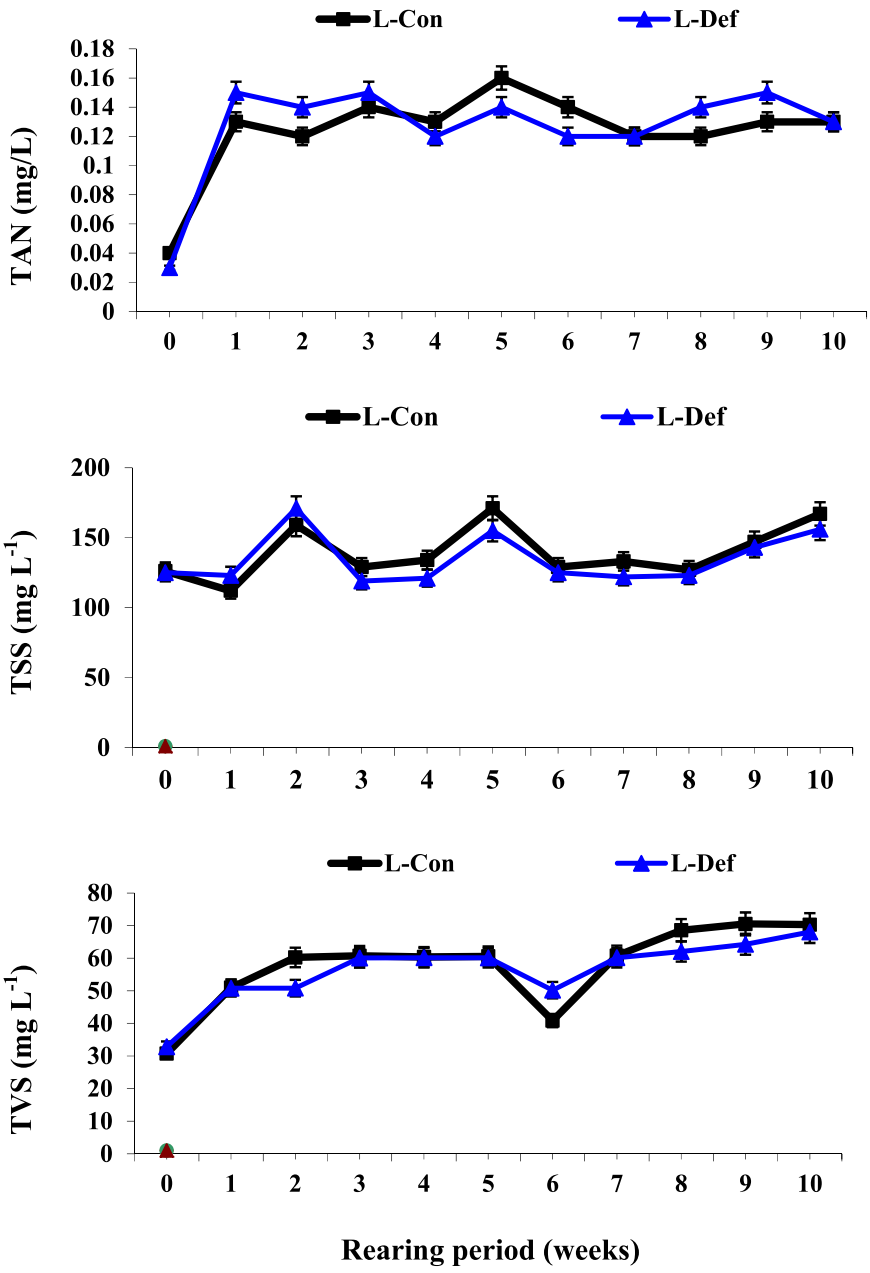


**Fig. 2.** Total ammonia-nitrogen (TAN), total suspended solids (TSS) and total volatile solids (TVS) levels of Nile tilapia (*O. niloticus*) fed the lysine diets for 10 weeks, n = 5; means ± SE.

dissolved oxygen (7.31 ± 0.51 mg $L^{-1}$); total suspended solids (129.81 ± 57.53 mg $L^{-1}$); total volatile solids (55.51 ± 23.81 mg $L^{-1}$); total ammonia-nitrogen (0.12 ± 0.07 mg $L^{-1}$) (Fig. 1 & 2).

### 3.2. Plankton abundance

For the two lysine groups, we found plankton species in the pond water as follows: Phytoplankton species counts (*$10^6$ $L^{-1}$) were calculated for the phyla Euglenophyta (unicellular algae – Euglenophyceae 3.29 ± 0.11); Bacillariophyta (diatoms - Bacillariophyceae 9.02 ± 2.7); Chlorophyta (green algae - Chlorophyceae 8.86 ± 0.16); Dinophyta (unicellular algae - Dinoflagellata 0.05 ± 0.01); and Cryptophyta (unicellular algae – Cryptophyceae 1.03 ± 0.22) (Table 2).

Zooplankton species counts (*$10^3$ $L^{-1}$) were calculated for the phyla Copepoda (Hexanauplia 0.84 ± 0.08); Rotifera (0.94 ± 0.48); Cladocera (Branchiopoda 0.34 ± 0.14); Protozoa (0.02 ± 0.01); and Cyanobacteria (blue-green algae - Cyanobacteria 8.86 ± 1.4) (Table 2).

The total phytoplankton concentration was 22.61 ± 12.54 * $10^6$ $L^{-1}$ and total zooplankton concentration was 2.11 ± 0.38 * $10^3$ $L^{-1}$, with no significant differences between the two groups ($P > 0.05$; Table 2).

### 3.3. Gut contents indices

The plankton selectivity index ranged from 1 to − 1, with statistically significant differences between groups. Nevertheless, the L-Def group had (+) positive significant values for all phyto and zooplankton phyla, except Cladocera, and was 0 for Chlorophyta. Meanwhile, L-Con group had (-) negative significant values for Euglenophyta, Chlorophyta and Copepoda (Fig. 3). Additionally, the plankton selectivity ratio (Ivlev's forage ratio) was similar ($P > 0.05$) between both groups, except a significantly higher ratio of Bacillariophyta was found with the L-Def group (Fig. 4).

**Table 2**
Plankton concentration $L^{-1}$ in the outdoor pond system for Nile tilapia *O. niloticus*, after 10 weeks.

| | Phylum | L-Con | L-Def | SEM | P-value |
|---|---|---|---|---|---|
| **Phytoplankton * $10^6$** | Cyanophyta | 8.86 | 10.26 | 2.92 | 0.694 |
| | Bacillarophyta | 9.02 | 11.72 | 5.40 | 0.339 |
| | Euglenophyta | 3.24 | 3.35 | 1.26 | 0.979 |
| | Dinoflagellata | 0.05 | 0.00 | 0.06 | 0.194 |
| | Chlorophyta | 8.86 | 9.02 | 2.88 | 0.975 |
| | Cryptophyta | 1.03 | 0.81 | 0.45 | 0.437 |
| | Total | 22.61 | 35.15 | 9.32 | 0.518 |
| **Zooplankton * $10^3$** | Cladocera | 0.34 | 0.21 | 0.21 | 0.500 |
| | Copepoda | 0.84 | 0.92 | 0.30 | 1.000 |
| | Rotifera | 0.94 | 1.42 | 0.27 | 0.887 |
| | Protozoa | 0.02 | 0.00 | 0.03 | 0.569 |
| | Total | 2.11 | 2.49 | 0.33 | 0.895 |

Data represented as means ± SE (n = 5) corresponding means are significantly different at $P < 0.05$.

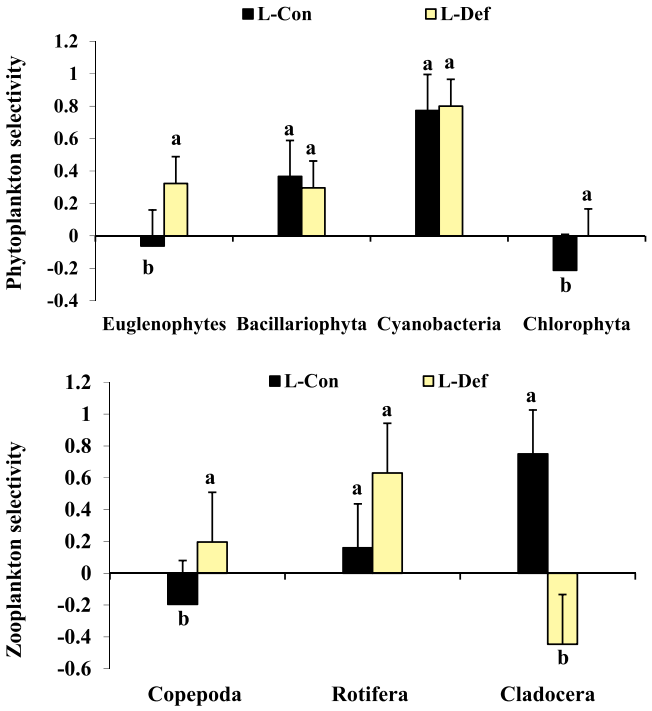


**Fig. 3.** Plankton selectivity (Ivlev index) by Nile tilapia, *O. niloticus,* fed a lysine reference diet (L-Con) and a lysine deficient diet (L-Def) after 10 weeks in ponds. Data represented as means ± SE (n = 5), $P > 0.05$.

At the end of the experiment, the numerical index (%) of plankton in the gut contents showed a significantly higher % of plankton in the L-Def group (48%), compared with the L-Con group (38%). Meanwhile, the numerical index (%) of feed in the fish gut was higher in the L-Con group, and there was no significant difference ($P > 0.05$) in the percentage of sand, plant parts and unidentified items between the two groups (Fig. 5).

### 3.4. Fish growth, organo-somatic indices, and fillet yield

Fish fed both groups had a statistically ($P > 0.05$) similar response in terms of both growth parameters and mortality. The final weight (g), fish yield (kg/pond), metabolic growth rate (g $kg^{-0.8}$ $day^{-1}$), and thermal growth coefficient ($°C^{-1}$) showed no significant differences between the groups (Table 3).

The impact of the lysine content in the diet on organo-somatic indices and fillet yield %, as markers of growth integrity, is summarised in Fig. 6. The condition factor, viscera-somatic indices, hepato-somatic indices, gonado-somatic indices, and fillet yield were not significantly different ($P > 0.05$) between diets.

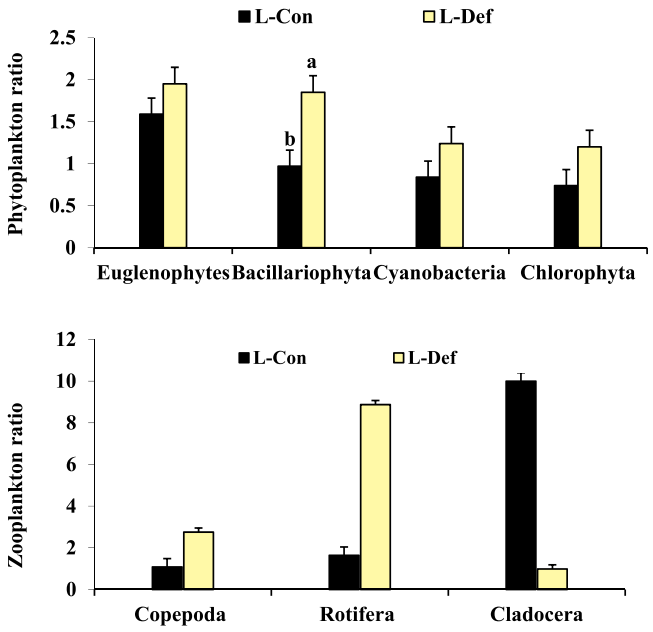


**Fig. 4.** Plankton selectivity ratio (Ivlev's forage ratio) of Nile tilapia, *O. niloticus,* fed a lysine reference diet (L-Con) and a lysine deficient diet (L-Def) after 10 weeks in ponds. Data represented as means ± SE (n = 5), $P < 0.05$.

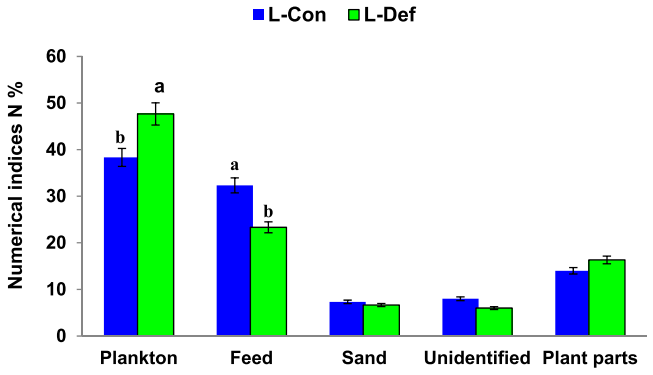


**Fig. 5.** Numerical indices (N%) of guts contents of Nile tilapia, *O. niloticus,* fed on lysine diets after 10 weeks in, n = 15; $P < 0.05$. Means with different superscript letter are significantly different ($P < 0.05$).

**Table 3**
Growth productive parameters of Nile tilapia, *O. niloticus,* fed a lysine reference diet (L-Con) and a lysine deficient diet (L-Def) after 10 weeks in ponds.

| Parameters | L-Con | L-Def | SEM | P-value |
|---|---|---|---|---|
| **Initial Weight (g)** | 17.4 | 17.04 | 1.70 | 0.751 |
| **Final Weight (g)** | 146.2 | 136.5 | 7.77 | 0.086 |
| **Weight gain (g)** | 129.85 | 119.46 | 10.44 | 0.361 |
| **Fish yield (kg $ha^{-1}$)** | 2281.45 | 2119.44 | 156.31 | 0.102 |
| **MGR (g $kg^{-0.8}$ $day^{-1}$)** | 20.1 | 19.37 | 1.57 | 0.483 |
| **TGC ($°C^{-1}$)** | 0.14 | 0.13 | 0.01 | 0.182 |
| **Fish Mortality (%)** | 2.42 | 2.36 | 1.89 | 0.944 |

Mean parameters including standard error of mean (SEM), n = 30; $P < 0.05$, MGR: the metabolic growth rate and TGC: the thermal growth coefficient.

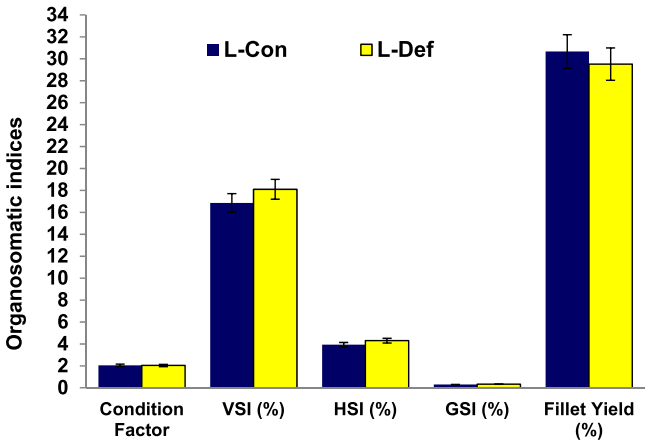


**Fig. 6.** Effect of the lysine diets on condition factor, organo-somatic indices, and fillet yield of Nile tilapia, *O. niloticus,* reared in outdoor ponds after 10 weeks. VSI = viscerosomatic indices; HSI = hepatosomatic indices; GSI = gonadosomatic indices, mean parameters including standard error (SE), n = 10, P < 0.05.

**Table 4**
Haematological parameters of Nile tilapia, *O. niloticus,* after 10 weeks fed on lysine reference diet (L-Con) and a lysine deficient diet (L-Def) in ponds.

| Parameters | L-Con | L-Def | SEM | P-value |
|---|---|---|---|---|
| **RBCs ($10^6$ mm$^{-3}$)** | 1.42 | 1.32 | 0.133 | 0.275 |
| **Haemoglobin (g dL$^{-1}$)** | 6.78 | 6.34 | 0.334 | 0.066 |
| **Haematocrit (%)** | 19.48 | 17.71 | 1.540 | 0.106 |
| **MCV (mm$^{-3}$)** | 138.01 | 138.80 | 2.494 | 0.630 |
| **MCH (pg)** | 47.74 | 48.03 | 3.659 | 0.487 |
| **WBCs ($10^3$ mm$^{-3}$)** | 62.44 | 66.91 | 12.84 | 0.596 |

Mean parameters including standard error of mean (SEM), n = 5; P < 0.05; RBC = Red blood cells; MCV = Mean corpuscular volume; MCH = Mean cell haemoglobin; WBC = White blood cells.

**Table 5**
Serum biochemical indices of Nile tilapia *O. niloticus* after 10 weeks fed on a lysine reference diet (L-Con) and a lysine deficient diet (L-Def) in ponds.

| Parameters | L-Con | L-Def | SEM | P-value |
|---|---|---|---|---|
| **ALT (U L$^{-1}$)** | 8.70 | 9.51 | 2.74 | 0.653 |
| **AST (U L$^{-1}$)** | 25.88 | 35.85 | 6.89 | 0.051 |
| **Alkaline phosphatase (U L$^{-1}$)** | 29.95 | 40.19 | 8.72 | 0.101 |
| **Total protein (g dl$^{-1}$)** | 3.57 | 3.44 | 0.20 | 0.298 |
| **Albumin (g dL$^{-1}$)** | 1.20 | 1.16 | 0.07 | 0.247 |
| **Globulin (g dL$^{-1}$)** | 2.37 | 2.28 | 0.16 | 0.422 |
| **Triglycerides (mg dL$^{-1}$)** | 296.30 | 295.71 | 9.67 | 0.919 |
| **Serum calcium (mg dL$^{-1}$)** | 11.26 | 11.96 | 0.69 | 0.154 |
| **Phosphorous (mg dL$^{-1}$)** | 8.42 | 9.40 | 1.78 | 0.405 |

Mean parameters including standard error of mean (SEM), n = 5; P < 0.05; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.

### 3.5. Haemato-biochemical parameters

Generally, RBC count, haemoglobin, haematocrit, MCV and WBC counts of Nile tilapia in the current experiment were not significantly different (P > 0.05) between groups (Table 4).

The lysine levels in the tested diets did not significantly influence (P > 0.05) in the serum biochemical parameters of Nile tilapia reared in fertilised ponds (Table 5).

### 3.6. Intestinal health

The intestinal morphology of fish subjected to both lysine diets is presented in Fig. 7. The intestinal cross-sections from the L-Con and L-Def groups showed numerous goblet cells in the proximal, middle, and distal gut, while the distal gut shows a partial serosal surface. The intestinal morphometry of the L-Con group displayed a significant increase (P < 0.05) in proximal villi length, and proximal and distal villi width (Table 6). However, there was significant decrease (P < 0.05) in length of the distal gut for submucosal layer and muscular layer. Meanwhile, 7 and 3 times higher goblet cell counts were observed in the proximal and mid gut, respectively, of the L-Def group, compared with the L-Con group. Furthermore, there were significant increases in distal villi length, mid villi width, and proximal lamina propria, as well as the distal section of the submucosal layer (μm), in the L-Def group, compared with the L-Con group (Table 6).

## 4. Discussion

The deficiency of dietary lysine at the same concentration has been shown to affect Nile tilapia growth and feed utilisation in clear-water tanks (Khalil et al., 2021). In the present study, we assessed the effect of dietary lysine deficiency on gut contents, plankton selectivity, metabolic growth, haemato-biochemical parameters, and intestinal morphometry of Nile tilapia raised in fertilised earthen ponds.

### 4.1. Water quality

There were significant changes in temp, $NO_3$, TSS and TVS during the experiment. The organic load in the water column increased and was variable, as shown by a high standard error of the mean for TVS, while the water transparency declined. Nevertheless, the average of physico-chemical water parameters throughout the culture period remained within recommended limits for optimum growth and performance of Nile tilapia in ponds (El-Sayed, 2019; Kord et al., 2021b).

### 4.2. Plankton abundance and selectivity

The total phytoplankton concentration in ponds and gut contents in the L-Def group had high positive phytoplankton selectivity values, which indicates preference for phytoplankton species, except for green algae, where the selectivity was close to 0. In the case of Nile tilapia, this suggests passive ingestion while filtering. Furthermore, the phytoplankton selectivity ratio for diatoms was significantly higher in the L-Def group than the L-Con group. These results agree with Nunn et al. (2007), who reported that fish select plankton with a high nutrient content when nutrient availability is low, to meet its dietary requirements. In addition, Shalloof, Khalifa (2009) found that Nile tilapia prefers diatoms over green algae in its feeding. The trend in the plankton concentration in the gut content could be explained by the preference of Nile tilapia for phytoplankton and zooplankton to meet feeding requirements (Yanuhar et al., 2018; Cunha et al., 2019). On the other hand, the total zooplankton concentration in ponds, and the L-Def group showed the highest positive selectivity on Copepoda and Rotifera. Meanwhile, Nile tilapia fed the L-Def diet avoided Cladocera. Similar results were reported by Abdel-Tawwab (2011); El-Sayed (2019), who suggested that Nile tilapia prefers zooplankton above plants ingredients. However, other reports suggest that Nile tilapia may switch between phytoplankton and zooplankton feeding depending on their nature and abundance in the ponds (Perschbacher, Stickney, 2017; Vasconcelos et al., 2018). In fed ponds, the role of natural food is relatively reduced, but due to the high stocking density, the absolute predation on the plankton community remains high. We observe however that in L-Def ponds, the fish searches more actively for some plankton species than in L-Con fed ponds. It does that to compensate for the lysine deficiency in the feed (Khalil et al., 2021). Also, Khatoon et al. (2009) revealed that diatoms contained higher amounts of essential AAs such as arginine, lysine,threonine, tyrosine, phenylalanine, and valine. It is worth noting that, the numerical indices (N%) of plankton was significantly higher in the gut of the L-Def group (48%) compared to the L-Con group (38%).

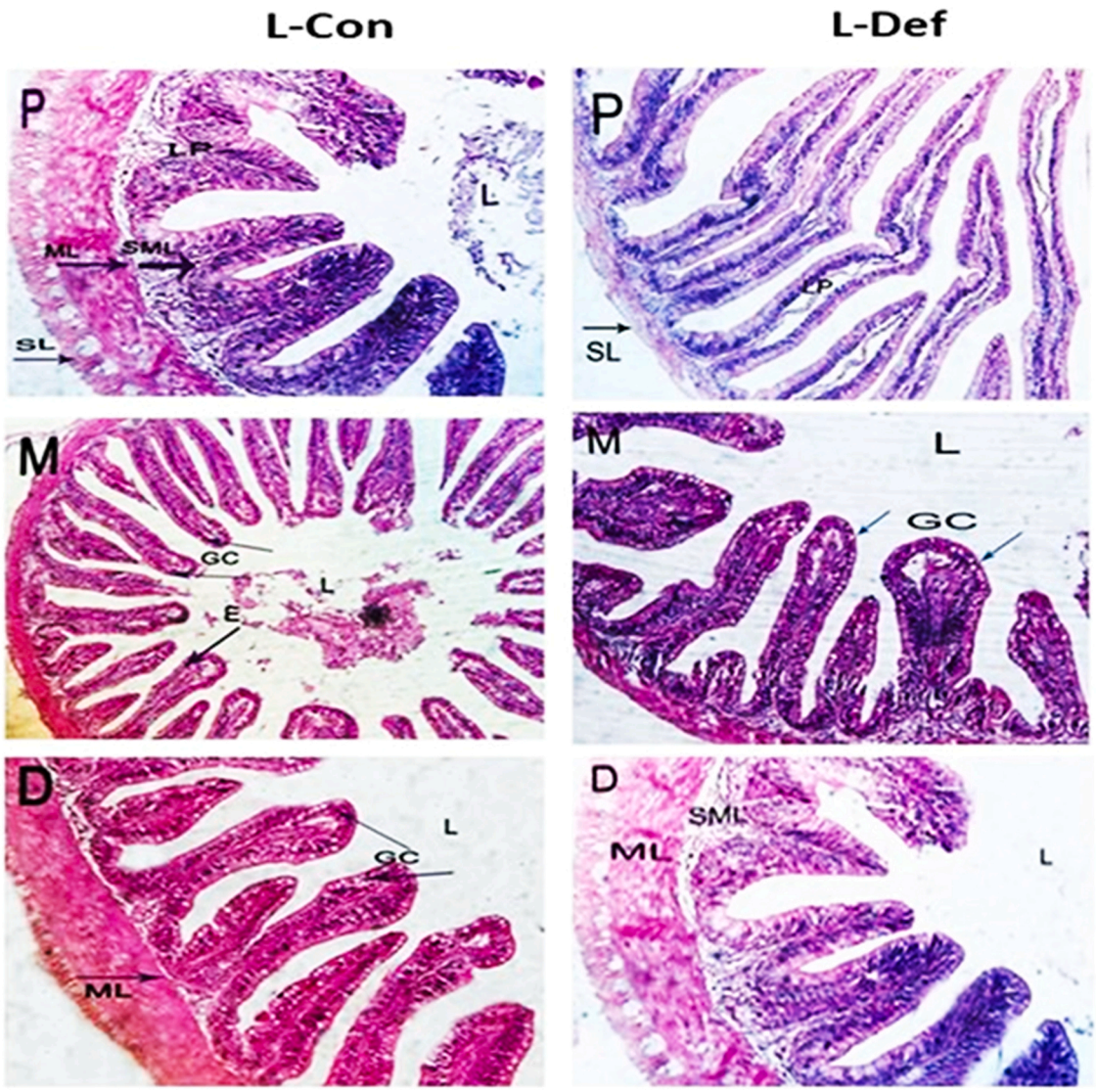


**Fig. 7.** Photomicrograph of cross-sections of the proximal (P), middle (M) and distal (D) intestine of Nile tilapia *O. niloticus* fed diets with different lysine levels and reared in outdoor ponds after 10 weeks, n = 10. Each cross-section is stained by Hematoxylin & Eosin (x 20) and shows GC (goblet cells), the length of intestinal villi (V), lumen (L), the thickness of the serosal layer (SL), lamina propria (LP), mucosa layer (mL) and submucosal layer (SML).

Meanwhile, the numerical indice of feed was significantly higher in the gut of the L-Con group. Agbabiaka (2012) and Budihastuti et al. (2013) found that Nile tilapia changed feeding behaviour according to the feed availability. According to Gbaguidi et al. (2016), Nile tilapia utilises many food sources, which to a high degree explains the high productions realised in ponds.

### 4.3. Fish growth and production

Growth, production and mortality (%) of a lysine-deficient group, reared in fertilised ponds, were similar with the control group. Studies have reported that the natural food in outdoor ponds contributes additional nutrients, including lysine, to the cultured fish (Li et al., 2020). Many essential micro-nutrients and biologically active components, such as amino acids, sterols, carotenoids, enzymes, fatty acids, trace elements, vitamins, chlorophyll, organic minerals, and antioxidants, are abundant in planktonic assemblages (Brown, 2002; Shields, Lupatsch, 2012; Molino et al., 2018; Ashour et al., 2020). If fish is able to eat a good selection of plankton species in the right amounts, then this provides all the nutrients and energy needed for maintenance and growth (Kovač et al., 2013; Napiórkowska-Krzebietke, 2017). The inclusion of lysine in fish plant protein diets is costly, and less needed in fertilised ponds, where part of the lysine was provided through the natural food web. In this experiment, dietary lysine deficiency did not result in decreased fish performance and survival. Further research is needed to elucidate how large the de novo provision of lysine through the food web in ponds can be, and which factors contribute to the de-novo in-situ lysine production in ponds.

### 4.4. Haemato-biochemical parameters

Haemato-biochemical parameters are indicators of fish welfare and stress tolerance (Seong et al., 2018; Khalil et al., 2019; Kord et al., 2021a). The results of the present experiment did not show a significant difference between the L-Con and L-Def groups in terms of the haemato-biochemical parameters of Nile tilapia raised in fertilised ponds. Generally, the levels of these parameters in this experiment agree with the reported ranges for healthy Nile tilapia (Hrubec et al., 2000; Davis et al., 2008). This confirms that the lack of lysine in the diet was compensated by grazing on the natural food in ponds, preventing

**Table 6**
Intestine morphometry sections of Nile tilapia, *O. niloticus,* after 10 weeks fed on lysine reference diet (L-Con) and a lysine deficient diet (L-Def) in ponds.

| Parameters | L-Con | L-Def | SEM | P-value |
|---|---|---|---|---|
| **Villi length (μm)** | | | | |
| Proximal | 768.66[a] | 462.10[b] | 72.27 | 0.003 |
| Mid | 511.17 | 588.78 | 23.31 | 0.089 |
| Distal | 227.16[b] | 334.29[a] | 25.39 | 0.004 |
| **Villi width (μm)** | | | | |
| Proximal | 70.60[a] | 44.66[b] | 3.69 | 0.001 |
| Mid | 22.06[b] | 49.33[a] | 2.02 | 0.013 |
| Distal | 76.66[a] | 56.67[b] | 4.28 | 0.023 |
| **Lamina propria (μm)** | | | | |
| Proximal | 51.67[b] | 92.29[a] | 9.31 | 0.001 |
| Mid | 89.78 | 68.42 | 6.67 | 0.110 |
| Distal | 106.55 | 93.62 | 5.61 | 0.295 |
| **Goblet cell count** | | | | |
| Proximal | 12.19[b] | 90.19[a] | 17.67 | 0.003 |
| Mid | 35.02[b] | 109.88[a] | 16.83 | 0.001 |
| Distal | 44.38 | 43.13 | 1.86 | 0.777 |
| **Submucosal layer (μm)** | | | | |
| Proximal | 50.05 | 54.02 | 3.40 | 0.617 |
| Mid | 22.96 | 20.97 | 1.89 | 0.654 |
| Distal | 14.30[b] | 50.25[a] | 8.50 | 0.004 |
| **Muscular layer (μm)** | | | | |
| Proximal | 39.55 | 42.83 | 3.69 | 0.706 |
| Mid | 49.75 | 62.06 | 5.26 | 0.287 |
| Distal | 88.14[b] | 96.58[a] | 4.96 | 0.045 0 |

Mean parameters including standard error (SE), n = 10; P < 0.05, Means with different superscript in the same row are significantly different.

deterioration of the haemato-biochemical parameters. Also, Lim et al. (2009) reported that lysine supplementation influenced haemoglobin, haematocrit and serum total immunoglobin in channel catfish and in grass carp (*Ctenopharyngodon idellus*) (Huang et al., 2021b). Deficiency in amino acids (AAs), including lysine, has been shown to affect fish health and immune function (Yaghoubi et al., 2017). Where the protein content of the aquatic plankton widely used for fish rearing is in the range of 50–57%, the most abundant essential amino acids (per 16 g N) were 9 essential and 8 non-essential amino acids, including: Lysine (9–11 g), Arginine (5–8 g), Leucine (8.0–9 g), Valine (5–6 g), Histidine (5–5.9 g), and Phenylalanine (5–5.5 g) (Ovie, Ovie, 2006; Kovač et al., 2013; Kolmakova and Kolmakov, 2019). The content of proteins varied in the range 30.6–50.7%; carbohydrates from 14.1% to 55.8%; lipids from 2.1% to 26.7% and phosphorus compounds from 8.0% to 21.0%, as recorded in natural estuarine phytoplankton (Ríos et al., 1998). There appears to be a strong link between plankton nutrients composition and plankton selectivity on growth parameters and fish health (Khalil et al., 2021). Li et al. (2011) and Lim et al. (2007) reported that distiller's dried grains with soluble as an alternative feed source did not affect Nile tilapia health when feeding lysine supplemented diets.

### 4.5. Intestinal health

The intestinal morphology may provide information on the effects of nutrition stressors (Rašković et al., 2013; Kord et al., 2021a). The intestine morphology of the L-Def group reared in fertilised ponds, showed partial serosal splitting. This might have led to lamina propria destruction, observed in the distal intestine, while the control diet only showed mild submucosal irritation. Typically, healthy intestine morphology (proximal, mid and distal) shows slender and discrete mucosal folds, well-vacuolated enterocytes, lamina propria of diminutive thickness and species-characteristic abundance and distribution of goblet cells (Rawles et al., 2013). In our study, we observed similar effects between the both treatments in the monitored intestinal morphology parameters. These results pointed to an improvement in the area of epithelial absorption, which contributes to increased feed utilisation, growth efficiency and intestine health. Similar results were reported by Kord et al. (2021a).

## 5. Conclusion

The results obtained from the present study implicate that Nile tilapia can change their plankton selectivity according to their feeding requirements and the plankton abundance. It is inferred the importance of plankton composition of lysine and its nutrient benefits in compensation of the lysine deficiency in plant aqua-feeds in fertilised ponds in terms of the growth performance and health status which is explained by the good performance on both diets.

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## CRediT authorship contribution statement

**H. Khalil:** Conceptualization, Methodology, Data curation, Supervision, Writing- Original draft preparation, Reviewing and Editing; **T. Momoh:** Data curation, Software, Writing, and Visualization; **D. Al-Kenawy:** Investigation, Monitoring and Reviewing; **A. Badr:** Diet formulation, Reviewing, and providing experimental feeds; **A. Roem:** Diet formulation, and providing experimental feeds; **R. Yossa:** Conceptualization, Reviewing and Monitoring; **J. Schrama:** Conceptualization, Reviewing, and Diet formulation; **M. Verdegem:** Conceptualization, Data curation, Supervision, Software, Validation, Writing-Reviewing and Editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability Statement

The data that support the findings of this research are available from the corresponding author.

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