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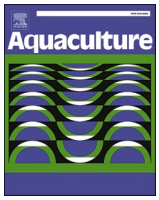

Dietary lysine requirement of genetically improved farmed tilapia (GIFT) juvenile reared in inland saline water of 10 ppt salinity

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ABSTRACT

A 60-day feeding trial was conducted to optimize the dietary lysine (LYS) requirement of juvenile genetically improved farmed tilapia (GIFT) reared in inland saline water (ISW) of 10 ppt salinity. Seven isonitrogenous (370 g crude protein/kg), isolipidic (80 g crude lipid/kg) and isocaloric (16.66 MJ digestible energy/kg) purified diets were formulated and prepared with graded LYS levels viz., 12.3 (L12.3), 14.7 (L14.7), 17.2 (L17.2), 19.6 (L19.6), 22.3 (L22.3), 24.8 (L24.8) and 27.2 g/kg (L27.2). GIFT juveniles (mean weight 3.16 ± 0.01 g, 315 numbers) were randomly allocated in triplicate into seven distinct groups following a completely randomized design. The juveniles were fed three times daily to apparent satiation level with the respective experimental diet. The results indicated that growth and nutrient utilization parameters were significantly ($p < 0.05$) altered by the various dietary LYS levels. These parameters exhibited an increasing trend from lowest dietary LYS level (12.3 g/kg) to 19.6 g/kg dietary LYS, and subsequent additional dietary LYS supplementation showed a declining trend. Significantly greater final weight, weight gain percentage, specific growth rate, protein efficiency ratio, apparent net protein retention and lower feed conversion ratio were found in the 19.6 g/kg LYS fed group than other groups. Different levels of LYS inclusion did not affect ($p > 0.05$) the body indices, survival and whole-body moisture and ash content of fish. An increment in dietary LYS up to 19.6 g/kg resulted in an increase in whole-body protein level and a decline in lipid level. The whole-body essential amino acid compositions of juveniles were not changed with various dietary LYS levels, whereas the non-essential amino acids increased with increasing dietary LYS levels up to 19.6 g/kg and decreased thereafter. Juveniles fed with high LYS containing diets exhibited higher aspartate aminotransferase and alanine aminotransferase activities, while digestive enzyme activities were unaffected. Serum protein, albumin, globulin, hemoglobin and white blood cell count changed with various dietary LYS levels. Based on broken-line linear and second-order polynomial regression analysis, the optimal dietary LYS requirements range was found to be 19.3–20.7 g/kg of the diet for GIFT juvenile reared in ISW of 10 ppt salinity.

1. Introduction

Salinization of land and groundwater is one of the emerging problems in the world and is gradually increasing with time (Lambers, 2003; Sandeep et al., 2013). The salt-affected lands and groundwater resources are unsuitable for agricultural activities (Machado and Serralheiro, 2017) and many countries around the world have successfully demonstrated euryhaline fish culture in such area (Fielder et al., 2001; Barman et al., 2005; Rahman et al., 2005; Jana et al., 2006; Partridge et al., 2006; Partridge and Lymbery, 2008; Kumar et al., 2009; Awal et al., 2016; Thomas et al., 2019; Singha et al., 2020; Jana et al., 2021; Thirunavukkarasar et al., 2021).

The success of feed-based intensive aquaculture dependents on the quality aquafeed used, where feed alone contributes over 50% of the whole operational expenditure (De Silva and Anderson, 1995). Among the various feed nutrients, protein is the extremely essential and costliest dietary nutrient that affects the growth performance of animals, including fish (Singh et al., 2006). Meanwhile, dietary protein beyond and below the optimum does not provide additional benefits in terms of growth. Moreover, excess dietary protein enhances nitrogenous wastes in the water and causes stress to the fish (Wilson and Halver, 1986). Therefore, dietary protein level needs to be optimized to reduce the feed

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costs and production cost as well. The optimum dietary protein requirement of Nile tilapia is ranged between 275 and 450 g/kg (Siddiqui et al., 1988; Abdel-Tawwab et al., 2010; Kpundeh et al., 2015; Castillo et al., 2017; Santos et al., 2020). Abdel-Tawwab et al. (2010) observed that dietary protein requirement of freshwater reared Nile tilapia varies with life stage and size, and reported that the protein requirement for fry (0.4–0.5 g initial body weight, IBW) and fingerling (17–22 g IBW) or juvenile (37–43 g IBW) was 450 and 350 g/kg, respectively. Similarly, Castillo et al. (2017) recommended that the dietary protein requirements of early fingerlings (2.01 g IBW), fingerlings (14.26 g IBW), juveniles (59.90 g IBW) and adults Nile tilapia reared in freshwater were 350, 325, 300 and 275 g/kg, respectively. Furthermore, Wu et al. (2021) observed variation in dietary protein requirement of GIFT under different salinities and found that GIFT reared in brackish water (8 ppt) displayed a higher protein requirement (377 g/kg) than GIFT reared in freshwater (327 g/kg).

The protein utilization efficiency of fish mostly depended on the proportion and ratio of the essential amino acids (EAAs) and non-essential amino acids (NEAAs) content of the feed (Akiyama et al., 1997). Both EAA and NEAA play important and multifaceted roles in the fish body, including growth regulation, nutrient metabolism, immune system management, reproductive development, etc. (Li et al., 2009; Andersen et al., 2016). However, all the EAAs must be included in the fish feed at an optimum requirement level, as they are not synthesized in the body or inadequately synthesized relative to the needs (NRC, 2011) and deficiency of these amino acids in fish diets can result in poor growth and low nutrient utilization (Andersen et al., 2016).

LYS is the first limiting essential amino acid in most feedstuffs incorporated in aquafeed, primarily when animal-based protein sources are substituted by plant protein sources (Mai et al., 2006). LYS is present in a high amount in fish muscle tissue, participates in the growth, maintenance of positive nitrogen balance and collagen synthesis (Sandell and Daniel, 1988; Michelato et al., 2016). Additionally, it has a vital role in carnitine synthesis, which helps in transport of long-chain fatty acids to the mitochondria for beta-oxidation (Dias et al., 2001). The addition of LYS to fish feed has shown several benefits, such as increased growth and feed efficiency (Sardar et al., 2009; Prabu et al., 2020), decreased excretion of ammonia and soluble phosphorus (Cao et al., 2012), improved gastrointestinal health (Li et al., 2009) and reduced body lipid levels (Furuya et al., 2012). It has been observed that LYS deficiency in feed results in lowered growth rate, appetite and protein utilization with fin erosion and high mortality in many fish (Mai et al., 2006; Khan and Abidi, 2011; NRC, 2011). Several studies were conducted to determine the LYS requirement of Nile tilapia reared in freshwater and the values were reported to be 14.3 g/kg (0.40 g IBW, Santiago and Lovell, 1988), 21.7–23.2 g/kg (0.98 g IBW, Takishita et al., 2009), 18.0 g/kg (1.12 g IBW, Bomfim et al., 2010), 15.2 g/kg (1.44 g IBW, Furuya et al., 2012), 18.0–19.5 g/kg (5.20 g IBW, Prabu et al., 2020), 14.4 g/kg (5.72 g IBW, Furuya et al., 2006), 14.2–14.6 g/kg (6.40 g IBW, Nguyen and Davis, 2016), 24.9 g/kg (9.01–11.09 g IBW, Ovie and Eze, 2010), 13.0–21.0 g/kg (12.00 g IBW, Liebert and Benkendorff, 2007), 15.6 g/kg (20.00 g IBW, Diogenes et al., 2016), 14.6 g/kg (27.49 g IBW, Michelato et al., 2016), 13.1 g/kg (86.62 g, IBW, Furuya et al., 2013), 14.2 g/kg (117.90 g IBW, Furuya et al., 2004) and 15.9 g/kg (158.78 g IBW, do Nascimento et al., 2020). This large difference in dietary LYS requirement is due to the differences in fish sizes and rearing conditions used by the investigators. Therefore, dietary LYS levels should be optimized taking into account the body size and rearing conditions of the fish for successful aquaculture.

Tilapia belongs to the family Cichlidae and is the second most important cultured group globally after carps (El-Sayed, 2006). Of the 70 tilapia species, Nile tilapia is one of the most cultivated tilapia species and contributes 83% of total tilapia production worldwide (FAO, 2018). The genetically improved farmed tilapia (GIFT) developed via a selective breeding program, grows faster and shows better feed utilization than the common strain of Nile tilapia (Ponzoni et al., 2011). GIFT can tolerate various environmental conditions, including salinity and high stocking density with high resistance to diseases (Suresh and Lin, 1992). Therefore, GIFT may be one of the most favorable fish species for cultivation in ISW. Few investigations have been conducted to assess the dietary LYS requirement of GIFT reared in freshwater (Prabu et al., 2020). However, no information is yet available regarding the LYS requirement for GIFT reared in coastal saline water as well as in ISW. Hence, the current study was performed to estimate the optimal dietary LYS requirement of GIFT juvenile reared in ISW of 10 ppt salinity.

2. Materials and methods

2.1. Experimental animal

Genetically improved farmed tilapia (GIFT) fry (avg. wt. 0.200 ± 0.001 g) was obtained from MM Hatcheries, Chhattisgarh, India, packed in a polythene bag filled with oxygen and transported to the regional centre of ICAR-Central Institute of Fisheries Education (CIFE), Rohtak, Haryana, India. Fry were shifted from polythene bags to 10,000 L cemented tanks containing aerated freshwater. Fish were kept for acclimatization in cemented tanks containing freshwater for 15 days and then in ISW (10 ppt salinity) for 15 days. Throughout the acclimatization periods, fry were fed with commercial diet (380 g crude protein/kg; 60 g lipid/kg; 16.49 MJ digestible energy/kg) three times a day to satiety level. Proper aeration was given in the tanks to sustain an adequate dissolved oxygen level. Later, for another 15 days these fish were familiarized to a purified diet (370 g crude protein/kg; 80 g lipid/kg; 16.65 MJ digestible energy/kg).

2.2. Formulation and preparation of experimental diets

Seven isonitrogenous (370 g crude protein/kg), isolipidic (80 g lipid/kg) and isocaloric (16.66 MJ digestible energy/kg) diets were formulated using purified ingredients (Table 1). The purified protein ingredients (casein and gelatin) were incorporated in such proportion that they contributed the minimum amount of LYS in the diet and crystalline L-LYS were supplemented in graded levels to produce different experimental diets with increments of 2.5 g LYS/kg. Other EAAs were supplemented to meet established amino acid requirements for Nile tilapia juvenile (NRC, 2011). A mixture of NEAAs was used to compensate the variable amounts of LYS in different experimental diets. Dietary protein (Singha et al., 2020) and lipid (Vijayakumar, 2020) levels were fixed based on earlier studies recommended for rearing GIFT juvenile in ISW at 10 ppt salinity.

All the pre-weighed ingredients except gelatin, crystalline amino acids, oil and additives were mixed properly with the addition of desired amount of water to prepare dough. Then, the dough was kept in a plastic bag and steam-cooked for 20 min. Concurrently, L-crystalline amino acids were weighed and properly mixed with warm water (80 °C) until completely dissolved, and the pH of the resulting mixture was raised to neutral with 6 N sodium hydroxide (Nose et al., 1974). The pre-weighed gelatin was added to the hot water to dissolve. Then, the dissolved gelatin was mixed with the dissolved amino acids according to Abidi and Khan (2007). Subsequently, the amino acid-gelatin mixture, oil and additives were thoroughly mixed to the cooked dough after cooling and dough was re-made. This dough was used to prepare pellets using a pelletizer fitted with a 2 mm die, which was oven-dried (45 °C) to achieve a moisture level below 100 g/kg and stored in the refrigerator until use.

2.3. Experimental design and management

For the assessment of LYS requirement, a sixty day's experiment was conducted at the indoor wet laboratory of regional centre, ICAR-CIFE, Rohtak, Haryana, India. The acclimated GIFT juveniles (mean weight 3.16 ± 0.01 g) were randomly distributed (fifteen fish per experimental

Table 1
Formulation and proximate composition (gram per kg, dry matter) of the different experimental diets used for feeding trial.

Ingredients	Diets[1]						
	L12.3	L14.7	L17.2	L19.6	L22.3	L24.8	L27.2
Casein	176.0	176.0	176.0	176.0	176.0	176.0	176.0
Gelatin	44.0	44.0	44.0	44.0	44.0	44.0	44.0
Starch	260.0	260.0	260.0	260.0	260.0	260.0	260.0
Dextrin	124.5	124.5	124.5	124.5	124.5	124.5	124.5
Cellulose	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Cod liver oil[2]	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Sunflower oil[3]	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin-Mineral mixture[4]	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Carboxymethylcellulose	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Butylated hydroxytoluene	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Betaine	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Choline chloride	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Stay C9[5]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
EAA mixture[6]	31.0	31.0	31.0	31.0	31.0	31.0	31.0
NEAA mixture[7]	186.0	183.5	181.0	178.5	176.0	173.5	171.0
Lysine	0.0	2.5	5.0	7.5	10.0	12.5	15.0
Cysteine	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Proximate composition							
Dry matter	929.0	932.2	931.6	931.3	931.6	928.8	929.9
Crude protein	371.6	372.3	375.2	368.7	373.0	370.2	369.8
Ether extract	81.3	83.8	81.7	80.8	81.1	79.4	82.6
Crude fiber	54.5	56.2	58.4	57.2	57.9	54.2	57.7
Nitrogen free extract	444.2	435.8	437.4	442.1	439.2	444.0	438.0
Total ash	48.4	52.0	47.2	51.1	48.8	52.2	51.9
Digestible energy (MJ/kg)	16.72	16.68	16.68	16.62	16.65	16.62	16.63

All purified ingredients procured from HiMedia Ltd., India.
Proximate composition are expressed as Mean ($n = 3$).
[1] Different experimental diet with graded levels of LYS (L12.3–12.3 g LYS from ingredient +0 g LYS supplementation in 1 kg diet; L14.7–12.2 g LYS from ingredient +2.5 g LYS supplementation in 1 kg diet; L17.2–12.2 g LYS from ingredient +5 g LYS supplementation in 1 kg diet; L19.6–12.1 g LYS from ingredient +7.5 g LYS supplementation in 1 kg diet; L22.3–12.3 g LYS from ingredient +10 g LYS supplementation in 1 kg diet; L24.8–12.3 g LYS from ingredient +12.5 g LYS supplementation in 1 kg diet; L27.2–12.2 g LYS from ingredient +15 g LYS supplementation in 1 kg diet).
[2] Procured from Sanofi India Ltd., India.
[3] Fortune Refined Sunflower Oil procured from local market of Mumbai, India.
[4] Composition of the Vitamin-mineral mixture (quantity/kg): Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin E, 750 mg; Vitamin K, 1000 mg; Ascorbic acid, 2500 mg; Vitamin B2, 2000 mg; Vitamin B6, 1000 mg; Vitamin B12, 6 mg; Calcium pantothenate, 2500 mg; Nicotinamide, 10 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 mg; Selenium, 125 mg.
[5] Stay-C9 (Vitamin C), DSM Nutritional Technologies, Mumbai, India.
[6] Essential amino acid mixture (EAA) (g/100 g): Arginine 13.11; Histidine 19.32; Isoleucine 5.66; Leucine 13.24; Methionine 7.76; Phenylalanine 8.61; Threonine 12.9; Tryptophan 3.80; Valine 15.6.
[7] Non-essential amino acid mixture (NEAA) (g/100 g): Alanine 13.24; Aspartic acid 22.03; Glycine 12.21; Glutamic acid 30.25; Proline 11.44; Serine 10.83.

unit) in seven distinct experimental groups viz., L12.3 (12.3 g LYS/kg of diet), L14.7 (14.7 g LYS/kg of diet), L17.2 (17.2 g LYS/kg of diet), L19.6 (19.6 g LYS/kg of diet), L22.3 (22.3 g LYS/kg of diet), L24.8 (24.8 g LYS/kg of diet) and L27.2 (27.2 g LYS/kg of diet) in triplicates following a completely randomized design. Circular tanks with a capacity of 300 L water (45 cm height and 92 cm diameter) were used for the experiment, and 220 L water volume was maintained in each tank throughout the experiment. During the experiment, fish were hand-fed daily three times (08:30, 12:30 and 16:30 h) to satiation level. Daily feed consumption was precisely recorded to determine feed intake. Fecal matter from each tank was regularly siphoned out with addition of same amount of siphoned ISW (10 ppt salinity) to maintain 220 L water volume. Round the clock aeration was provided to each experimental tank with the help of an air blower to keep the dissolved oxygen level within the optimum range.

2.4. Water quality parameters

Water quality indices were analyzed every alternative day and maintained within the optimum range in each experimental unit throughout the experimental period. The digital water thermometer was used to test water temperature (Fisher Scientific, USA) and maintained uniform temperature in each experimental unit with a thermostat heater. The water salinity and pH were checked using an analog refractometer (Fisher Scientific, USA) and an electronic pH meter (HANNA Instruments, Singapore), respectively. The portable DO meter (Lutron, Taiwan) was used to determine the dissolved oxygen level. Total alkalinity, total hardness, the concentration of calcium and magnesium ions were determined according to the standard methods (APHA, 2005). The Spectroquant water test kit (Merck Millipore, Germany) was employed to quantify the ammonia-N, nitrite-N and nitrate-N in water of experimental units. The digital flame photometer (Panomex laboratory instrument, India) was used to measure the concentration of potassium ions, and vapor pressure osmometer (VAPRO®, Germany) to measure osmolality.

2.5. Sampling

The overall biomass of the each experimental unit was recorded at the start and end of the trial to assess the growth indices. Five fish were taken from each experimental unit before final sampling for whole body proximate and amino acid composition analysis. Experimental fish were not fed 24 h before the sampling. Eight fish were collected from each experimental unit for final sampling and anesthetized with clove oil (50 μL/L). Liver and viscera were collected from three fish and weighed separately to calculate body indices. The other three fish were dissected to collect intestine, liver and muscle and kept in chilled sucrose solution (0.25 M). For enzyme assays, these tissues were homogenized using an

electronic homogenizer, collected the homogenate in a 15 mL conical centrifuge tube and stored at −20 °C. Blood was drawn from an anesthetized juvenile of each experimental unit and put in an EDTA coated tube to analyze hematological parameters. Blood was collected from another anesthetized fish in eppendorf tubes and allowed to clot at ambient temperature for serum collection. Then clotted blood tube was centrifuged at 2800*g* for 10 min, and serum (yellow straw colored supernatant) was transferred to another eppendorf tube and stored at −20 °C until used for the serum biochemical analysis.

2.6. Proximate and amino acid composition of the diets and fish whole body

The proximate composition of the diets and whole body of fish was estimated following the standard methods of AOAC (1995). The moisture, crude protein, lipid (ether extract), crude fiber and total ash were analyzed using the Kjeldahl method (Kelplus, Pelican Equipments, India), Solvent extraction method (SOCS plus, Pelican Equipments, India), acid and alkali digestion method (FiberTech, Tulin equipment, India) and muffle furnace (HIPL-022, Hexatec instruments, India), respectively. The nitrogen-free extract of the feed sample was computed as 1000 – [crude protein (g/kg) + ether extract (g/kg) + crude fiber (g/kg) + total ash (g/kg)], while the total carbohydrate of the whole body of fish was computed as 1000 – [moisture (g/kg) + crude protein (g/kg) + crude lipid (g/kg) + total ash (g/kg)]. The digestible energy (MJ/kg) of the diets was calculated according to Halver (1976). Amino acid composition of the diets and fish whole body samples were estimated using an automatic amino acid analyzer (Biochrom 30^{+}, United Kingdom) after hydrolyzing with 6 N hydrochloric acid in a dry block heater at 110 °C for 24 h (Kader et al., 2010).

2.7. Growth and nutrient utilization parameters

Weight gain (WG), weight gain percentage (WG%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein retention (ANPR) were calculated according to the formula given below:

i. WG (g) = final body weight - initial body weight
ii. WG% = [(final body weight - initial body weight)/initial body weight] × 100
iii. SGR (%/day) = [(Ln final body weight - Ln initial body weight)/experiment duration in days] × 100
iv. FCR = feed consumption (g)/ weight gain (g)
v. PER = weight gain (g)/protein consumption (g)
vi. ANPR (%) = [(final body weight × final body protein) - (initial body weight × initial protein content)]/protein consumption (g) × 100

2.8. Body indices and survival

Hepatosomatic index (HSI), viscerosomatic index (VSI) and survival percentage were calculated by the following formulas:

i. HSI (%) = (liver weight/fish weight) × 100
ii. VSI (%) = (viscera weight/fish wet weight) × 100
iii. Survival (%) = (number of fish harvested /number of fish stocked) × 100

2.9. Digestive and metabolic enzyme assay

The protein content of intestine, liver and muscle tissue homogenates was assessed by Bradford method (Bradford, 1976). The estimated values of tissue protein were used to calculate enzyme activities. The intestinal protease, amylase and lipase activities were determined by the method suggested by Drapeau (1976), Rick and Stegbauer (1974) and Cherry and Crandall (1932), respectively. The liver and muscle aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities of experimental fish were evaluated according to the method defined by Wooten (1964).

2.10. Serum biochemical and hematological parameters

Glucose level was estimated from serum samples using a Glucose Kit (Sigma Aldrich, USA) following the method given by Trinder (1969). The total serum protein was estimated by the Total Protein Kit (Sigma Aldrich, USA) based on the method described by Bradford (1976), whereas Bromocresol Green Albumin Assay Kit (Sigma Aldrich, USA) was used to evaluate the albumin content. Serum globulin level was computed by deducting albumin from serum total protein, and albumin was divided by globulin to compute the albumin:globulin ratio (A/G ratio). The hemoglobin concentration, red blood cell (RBC) and white blood cell (WBC) count were estimated from blood samples using an automated hematology analyzer (Sysmex XP-300™, USA).

2.11. Statistical analysis

All data were statistically examined using the IBM SPSS program software (SPSS, 22). All experimental data (except feed composition and water quality parameter) were subjected to one-way ANOVA with polynomial and quadratic contrast analysis, and post-hoc under DMRT was done to test overall, linear and quadratic trend at 5% probability level ($p < 0.05$), whereas proximate and amino acid composition of feed and water quality data were subjected to DMRT to identify significant differences at a 5% probability level ($p < 0.05$). The value of WG and FCR was fitted in the broken-line linear (Robbins et al., 2006) and second-order polynomial (Jobling, 1988) regression analysis model to assess the optimum dietary LYS requirement of GIFT juvenile.

3. Results

3.1. Proximate and amino acid composition of experimental diet

The proximate composition analysis revealed that all the diets were isonitrogenous, isolipidic and isocaloric (Table 1). The dry matter, crude protein, ether extract, crude fiber, nitrogen-free extract, total ash and digestible energy of various experimental diets were within the range from 928.8 to 932.2 g/kg, 368.7–375.2 g/kg, 79.4–83.8 g/kg, 54.2–58.4 g/kg, 435.8–444.2 g/kg, 47.2–52.2 g/kg and 16.62–16.72 MJ/kg, respectively. All EAAs except LYS content of various experimental diets were found to be comparable with those recommended by NRC (2011) for the diet of Nile tilapia juvenile (Table 2). The LYS levels in the experimental diets were found to be at graded levels such as 12.3, 14.7, 17.2, 19.6, 22.3, 24.8 and 27.2 g LYS/kg.

3.2. Water quality parameters

No significant changes ($p > 0.05$) were noticed in water quality indices throughout the experiment (Table 3). Temperature, salinity, pH, dissolved oxygen, total alkalinity, total hardness, ammonia-N, nitrite-N, nitrate-N, calcium, magnesium, potassium and osmolality were found to be in the range of 27.7–28.3 °C, 9.97–10.27 ppt, 7.60–8.07, 5.57–6.17 mg/L, 256.33–269.67 mg/L, 2809–2888 mg/L, 0.021–0.027 mg/L, 0.002–0.004 mg/L, 0.032–0.036 mg/L, 349.33–362.33 mg/L, 452.0–472.7 mg/L, 13.2–14.4 mg/L and 287.67–295.00 mOsmole/kg, respectively.

3.3. Growth and nutrient utilization

The growth and nutrient utilization of GIFT juveniles were significantly ($p < 0.05$) affected overall, linearly and quadratically by various dietary LYS levels (Table 4). The FW, WG, WG%, SGR, PER and ANPR

Table 2
Amino acid composition (gram per kg, dry weight basis) of the different experimental diets used for feeding trial.

Amino acids	Diets[1]						
	L12.3	L14.7	L17.2	L19.6	L22.3	L24.8	L27.2
Essential amino acids							
Arginine	12.1	12.0	12.3	12.2	12.1	12.1	12.3
Histidine	9.8	10.1	10.0	10.1	9.9	10.1	9.8
Leucine	19.1	19.4	19.2	19.1	19.0	19.1	19.3
Isoleucine	10.2	10.1	10.0	10.1	10.3	10.1	10.4
Lysine	12.3	14.7	17.2	19.6	22.3	24.8	27.2
Methionine	6.8	7.1	6.9	6.8	7.0	7.2	7.1
Phenylalanine	11.3	11.1	11.0	11.3	11.2	11.1	11.3
Threonine	10.9	10.8	11.0	10.6	10.7	10.9	11.0
Tryptophan	3.2	3.1	3.2	3.4	3.2	3.1	3.3
Valine	14.6	14.5	14.3	14.6	14.7	14.4	14.6
Non-essential amino acids							
Alanine	27.8	27.4	27.1	26.9	26.5	26.2	25.4
Aspartic acid	44.1	43.7	43.3	42.8	42.3	41.8	41.3
Cystine	3.1	3.0	3.3	3.1	3.0	3.2	3.3
Glutamic acid	69.4	68.8	68.0	67.3	66.9	66.3	65.6
Glycine	28.2	28.0	27.6	27.5	27.2	26.9	26.6
Proline	32.3	32.2	31.9	31.7	31.3	31.1	30.8
Serine	23.3	23.1	22.7	22.4	22.2	21.8	21.5
Tyrosine	5.1	5.3	5.1	5.1	5.3	5.2	5.3

All values are expressed as Mean (n = 3).
[1] Different experimental diet with graded levels of LYS (L12.3–12.3 g LYS from ingredient +0 g LYS supplementation in 1 kg diet; L14.7–12.2 g LYS from ingredient +2.5 g LYS supplementation in 1 kg diet; L17.2–12.2 g LYS from ingredient +5 g LYS supplementation in 1 kg diet; L19.6–12.1 g LYS from ingredient +7.5 g LYS supplementation in 1 kg diet; L22.3–12.3 g LYS from ingredient +10 g LYS supplementation in 1 kg diet; L24.8–12.3 g LYS from ingredient +12.5 g LYS supplementation in 1 kg diet; L27.2–12.2 g LYS from ingredient +15 g LYS supplementation in 1 kg diet).

significantly increased ($p < 0.05$) with increasing the LYS level in the diets up to 19.6 g/kg and subsequently, surplus dietary LYS inclusion resulted in a decreasing trend of the above parameters. Among all the experimental groups, the group fed with 12.3 g/kg LYS in diet had lowest FW, WG, WG%, SGR, PER and ANPR. Overall and quadratic trend of feed intake (FI) exhibited a significant ($p < 0.05$) change between different experimental groups. FI of L19.6 group was greater ($p < 0.05$) than that of L12.3 and L27.2 groups, but similar ($p > 0.05$) to other groups. Moreover, similar FI was found among L12.3, L14.7, L24.8 and L27.2 groups. The FCR significantly ($p < 0.05$) declined with increasing the LYS level in the diets up to 19.6 g/kg, thereafter remained constant. The significantly ($p < 0.05$) lowest FCR was recorded in L12.3 group.

Fitting the WG in broken-line linear and second-order polynomial regression model, the optimal LYS requirement in the diet of GIFT juvenile was determined as 19.4 and 20.1 g/kg of diet, respectively (Fig. 1) and based on FCR, the optimal LYS requirement in the diet was found to be 19.3 and 20.7 g/kg of diet, respectively (Fig. 2). Thus, the overall optimal LYS requirement in the diet of GIFT juvenile reared in ISW ranged between 19.3 and 20.7 g/kg of diet.

3.4. Body indices and survival

The HSI and VSI was unaffected ($p > 0.05$) by different dietary LYS levels. No mortality was recorded in any of the test groups (Table 4).

3.5. Proximate and amino acid composition of fish whole-body

Overall, linear and quadratic trends of whole body protein and lipid contents varied ($p < 0.05$) with different LYS levels in the feeds, but moisture, total carbohydrate and total ash contents of the different groups remained unchanged (Table 5). The protein level significantly ($p < 0.05$) elevated with increasing LYS level up to 19.6 g/kg of diet, and thereafter it remained constant or declined. The lowest LYS fed group (12.3 g/kg) exhibited the lowest protein level. The whole body lipid level followed the opposite trend of crude protein level, where lipid level was diminished ($p < 0.05$) with the increasing LYS level in the diet up to 19.6 g/kg, and thereafter it was either similar or declined.

The fish whole body amino acid composition of different dietary LYS fed groups is shown in Table 5. The EAA composition of the different groups were not affected ($p > 0.05$) by feeding various LYS levels; although the NEAA (alanine, aspartic acid, glutamic acid and serine) levels were increased ($p < 0.05$) with the increasing dietary LYS level up to optimal (19.6 g LYS/kg), thereafter it declined.

3.6. Digestive and metabolic enzyme activities

The protease, amylase and lipase activities were not affected ($p > 0.05$) by different dietary LYS levels, but the overall, linear and quadratic trends of liver and muscle AST and ALT activities were significantly ($p < 0.05$) affected due to different dietary LYS levels (Table 6). The liver AST activity of L22.3 group was higher ($p < 0.05$) than that of L12.3, L14.7 and L17.2 groups and similar ($p > 0.05$) to L19.6, L24.8 and L27.2 groups. On the other hand, L19.6 and L22.3 groups displayed significantly ($p < 0.05$) elevated muscle AST activity than L12.3 and L14.7 groups. Liver and muscle ALT activities were

Table 3
Water quality parameters of different experimental groups during the experimental period of 60 days.

Parameters	Diets[1]						
	L12.3	L14.7	L17.2	L19.6	L22.3	L24.8	L27.2
Temperature	28.10 ± 0.38	27.83 ± 0.32	28.03 ± 0.42	27.70 ± 0.53	28.03 ± 0.45	27.87 ± 0.43	28.30 ± 0.46
Salinity (ppt)	10.07 ± 0.15	9.97 ± 0.23	10.13 ± 0.17	10.00 ± 0.26	10.27 ± 0.23	10.10 ± 0.26	10.02 ± 0.31
pH	7.90 ± 0.21	8.07 ± 0.26	7.80 ± 0.40	8.03 ± 0.33	7.60 ± 0.25	8.03 ± 0.24	7.87 ± 0.34
Dissolve oxygen (mg/L)	5.63 ± 0.32	6.03 ± 0.18	6.00 ± 0.35	6.17 ± 0.15	6.07 ± 0.26	5.57 ± 0.37	5.87 ± 0.48
Total alkalinity (mg/L)	260.00 ± 9.29	263.67 ± 6.06	256.33 ± 4.91	265.00 ± 14.57	269.67 ± 7.51	266.33 ± 5.04	262.33 ± 9.94
Total hardness (mg/L)	2818.00 ± 28.18	2846.67 ± 57.98	2820.33 ± 30.75	2888.67 ± 60.95	2809.00 ± 64.02	2822.33 ± 91.36	2863.67 ± 45.48
Ammonia nitrogen (mg/L)	0.027 ± 0.003	0.024 ± 0.004	0.023 ± 0.002	0.021 ± 0.002	0.024 ± 0.003	0.021 ± 0.004	0.026 ± 0.003
Nitrite (mg/L)	0.004 ± 0.000	0.003 ± 0.000	0.002 ± 0.000	0.004 ± 0.000	0.002 ± 0.000	0.003 ± 0.000	0.002 ± 0.0000
Nitrate (mg/L)	0.033 ± 0.014	0.034 ± 0.011	0.033 ± 0.021	0.036 ± 0.013	0.034 ± 0.016	0.037 ± 0.012	0.032 ± 0.022
Calcium (mg/L)	354.33 ± 6.69	349.33 ± 14.72	351.33 ± 7.69	362.00 ± 13.65	336.33 ± 13.98	354.67 ± 15.72	362.33 ± 2.96
Magnesium (mg/L)	464.67 ± 4.91	459.00 ± 14.22	464.67 ± 6.44	472.67 ± 10.04	452.00 ± 12.49	467.00 ± 15.01	470.67 ± 4.37
Potassium (mg/L)	13.67 ± 1.25	13.20 ± 1.70	13.63 ± 0.44	14.40 ± 0.49	13.63 ± 0.61	14.17 ± 1.00	14.07 ± 0.91
Osmolality (mOsmole/kg)	290.67 ± 4.33	290.33 ± 7.62	290.33 ± 2.96	295.00 ± 5.57	291.00 ± 5.13	294.67 ± 5.21	287.67 ± 9.91

All values are expressed as Mean ± SE (n = 3).
[1] Different experimental diet with graded levels of LYS (L12.3–12.3 g LYS from ingredient +0 g LYS supplementation in 1 kg diet; L14.7–12.2 g LYS from ingredient +2.5 g LYS supplementation in 1 kg diet; L17.2–12.2 g LYS from ingredient +5 g LYS supplementation in 1 kg diet; L19.6–12.1 g LYS from ingredient +7.5 g LYS supplementation in 1 kg diet; L22.3–12.3 g LYS from ingredient +10 g LYS supplementation in 1 kg diet; L24.8–12.3 g LYS from ingredient +12.5 g LYS supplementation in 1 kg diet; L27.2–12.2 g LYS from ingredient +15 g LYS supplementation in 1 kg diet).

Table 4
Growth, feed and nutrient utilization, body indices and survival of GIFT juvenile reared in inland saline water of 10 ppt salinity and fed with graded dietary lysine levels for period of 60 days.

Parameters	Diets[1]							SEM[2]	Contrast analysis		
	L12.3	L14.7	L17.2	L19.6	L22.3	L24.8	L27.2		*p values*		
									Overall	Linear	Quadratic
Initial mean weight (g)	3.16	3.15	3.16	3.16	3.15	3.16	3.16	0.003	0.798	0.533	0.717
Final mean weight (g)	11.26^{a}	12.06^{b}	12.82^{cd}	13.47^{e}	13.10^{d}	12.74^{c}	12.49^{c}	0.154	0.001	0.001	0.001
Weight gain (g)	8.10^{a}	8.94^{b}	9.66^{cd}	10.31^{e}	9.95^{d}	9.58^{c}	9.33^{c}	0.153	0.001	0.001	0.001
Weight gain (%)	256.52^{a}	284.18^{b}	305.69^{cd}	326.38^{e}	315.80^{de}	303.45^{c}	294.95^{c}	4.856	0.001	0.001	0.001
Specific growth rate (%/day)	2.12^{a}	2.24^{b}	2.33^{cd}	2.42^{e}	2.38^{de}	2.32^{c}	2.29^{bc}	0.021	0.001	0.001	0.001
Feed intake (g/fish)	14.32^{a}	14.69^{abc}	14.94^{bc}	15.25^{c}	15.03^{bc}	14.74^{abc}	14.49^{ab}	0.085	0.024	0.440	0.001
Feed conversion ratio	1.77^{c}	1.64^{b}	1.55^{a}	1.48^{a}	1.51^{a}	1.54^{a}	1.56^{ab}	0.022	0.001	0.001	0.001
Protein efficiency ratio	1.52^{a}	1.64^{b}	1.72^{bc}	1.83^{d}	1.78^{cd}	1.76^{cd}	1.74^{cd}	0.023	0.001	0.001	0.001
Apparent net protein retention (%)	22.96^{a}	25.39^{b}	27.86^{c}	30.47^{e}	29.32^{de}	28.59^{cd}	27.86^{c}	0.543	0.001	0.001	0.001
Hepatosomatic index (%)	1.13	1.12	1.10	1.07	1.08	1.10	1.10	0.006	0.303	0.130	0.052
Viscerosomatic index (%)	9.37	9.40	9.27	8.99	9.06	9.14	9.30	0.080	0.816	0.453	0.275
Survival (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	–	–	–	–

All values are expressed as Mean (n = 3); Mean values in the same row with different superscripts differ significantly at 5% probability level ($p < 0.05$).
[1] Different experimental diet with graded levels of LYS (L12.3–12.3 g LYS from ingredient +0 g LYS supplementation in 1 kg diet; L14.7–12.2 g LYS from ingredient +2.5 g LYS supplementation in 1 kg diet; L17.2–12.2 g LYS from ingredient +5 g LYS supplementation in 1 kg diet; L19.6–12.1 g LYS from ingredient +7.5 g LYS supplementation in 1 kg diet; L22.3–12.3 g LYS from ingredient +10 g LYS supplementation in 1 kg diet; L24.8–12.3 g LYS from ingredient +12.5 g LYS supplementation in 1 kg diet; L27.2–12.2 g LYS from ingredient +15 g LYS supplementation in 1 kg diet).
[2] SEM, average standard error of means.

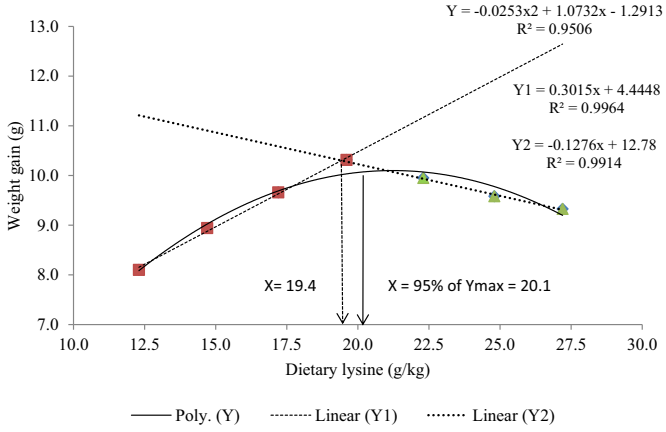

Fig. 1. Broken-line linear (dash line) and second-order polynomial (solid line) regression analysis for optimization of dietary lysine requirement in relation to weight gain (g) of GIFT juvenile reared in inland saline water of 10 ppt salinity and fed with graded dietary lysine levels for period of 60 days.

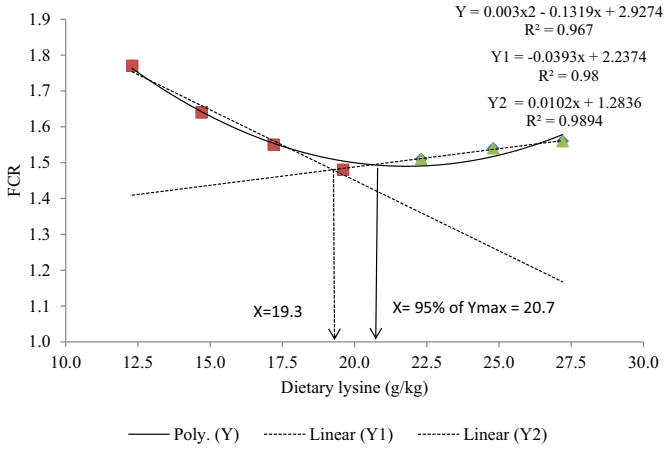

Fig. 2. Broken-line linear (dash line) and second-order polynomial (solid line) regression analysis for optimization of dietary lysine requirement in relation to feed conversion ratio (FCR) of GIFT juvenile reared in inland saline water of 10 ppt salinity and fed with graded dietary lysine levels for period of 60 days.

elevated ($p < 0.05$) with the increasing LYS level in diet up to 19.6 g/kg, and thereafter it declined.

3.7. Serum biochemical parameters

Serum total protein, albumin and globulin were significantly ($p < 0.05$) altered with overall, linear and quadratic trends in different groups (Table 7). Serum total protein and globulin levels were increased ($p < 0.05$) with the increasing LYS level in diet up to 19.6 g/kg, and thereafter it declined. Fish fed with 19.6 g LYS/kg had elevated ($p < 0.05$) albumin level than that of L12.3 and L14.7 groups and similar ($p > 0.05$) with other groups. On the other hand, serum glucose level and A:G ratio did not changed ($p > 0.05$) with feeding of various LYS levels.

3.8. Hematological parameters

Feeding graded levels of LYS resulted in significant ($p < 0.05$) alteration in blood hemoglobin level and WBC count in overall, linearly and quadratically, but did not affect RBC count (Table 7). Hemoglobin level and WBC count enhanced ($p < 0.05$) with the increasing dietary LYS level up to 24.8 g/kg and declined thereafter. There was no significant ($p > 0.05$) change in hemoglobin level among L19.6, L22.3 and L24.8 groups and WBC count among L22.3 and L24.8 groups.

4. Discussion

In the current study, water quality did not cause any adverse effect on fish growth as these were found within the acceptable limit for tilapia culture. Tilapia is a predominantly freshwater species; however GIFT strain of Nile tilapia can tolerate a wide range of salinity variations. Optimal growth of Nile tilapia can be achieved in water salinity ranging from 0 to 19 ppt (El-Leithy et al., 2019), and 10 ppt salinity level in our study was within this range for GIFT culture. The ideal water temperature required for GIFT rearing ranges from 22 to 30 °C (Qiang et al., 2013), and the water temperature range of 27.7 to 28.3 °C in our study was within this range. The dissolved oxygen level was maintained within an appropriate range throughout the experiment (Abdel-Tawwab et al., 2015). The water pH during the whole experimental period ranged from 7.60 to 8.07, which was found between the optimum limit for Nile

Table 5
Whole body proximate (g per kg, wet weight basis) and amino acid composition (g per kg, dry weight basis) of GIFT juvenile reared in inland saline water of 10 ppt salinity and fed with graded dietary lysine levels for period of 60 days.

Proximate composition	Diets[1]							SEM[2]	Contrast analysis		
	L12.3	L14.7	L17.2	L19.6	L22.3	L24.8	L27.2		*P values*		
									Overall	Linear	Quadratic
Moisture	726.36	730.88	725.85	725.58	730.76	723.46	729.53	1.466	0.822	0.985	0.853
Crude protein	152.66^{a}	155.86^{ab}	160.65^{cd}	164.08^{d}	163.38^{cd}	161.44^{cd}	159.44^{bc}	0.933	0.001	0.001	0.001
Crude lipid	77.84^{d}	74.55^{c}	70.45^{b}	66.49^{a}	66.69^{a}	69.16^{ab}	71.47^{b}	0.902	0.001	0.001	0.001
Total carbohydrate	12.05	8.48	10.26	9.85	8.24	12.40	8.48	1.321	0.977	0.822	0.837
Total ash	31.08	30.23	32.79	34.00	30.92	33.54	31.08	0.631	0.650	0.617	0.325
Essential amino acids											
Arginine	41.26	40.88	40.93	40.73	40.92	41.17	40.97	0.115	0.940	0.767	0.547
Histidine	10.19	9.74	10.07	10.11	10.32	10.12	10.18	0.132	0.970	0.654	0.947
Leucine	39.20	38.73	39.03	38.58	40.13	39.74	40.10	0.291	0.726	0.202	0.539
Isoleucine	22.40	22.24	22.29	21.93	22.60	22.75	22.53	0.161	0.914	0.508	0.608
Lysine	41.88	41.80	42.51	42.68	42.58	42.33	42.26	0.137	0.565	0.187	0.249
Methionine	14.21	14.36	14.45	14.49	14.35	14.39	14.37	0.053	0.910	0.595	0.328
Phenylalanine	20.16	19.91	20.01	20.17	20.96	20.52	20.98	0.201	0.725	0.146	0.694
Threonine	22.31	22.26	22.36	22.38	22.42	22.39	22.22	0.059	0.980	0.956	0.464
Tryptophan	5.26	5.28	5.30	5.32	5.26	5.28	5.23	0.030	0.996	0.763	0.588
Valine	24.52	24.76	24.63	24.97	25.18	24.88	24.70	0.093	0.615	0.343	0.194
Non-essential amino acids											
Alanine	33.39^{a}	34.05^{ab}	34.71^{abc}	35.96^{c}	35.22^{bc}	34.60^{abc}	34.21^{ab}	0.227	0.035	0.121	0.003
Aspartic acid	37.56^{a}	37.99^{ab}	38.21^{ab}	39.93^{c}	39.76^{c}	39.09^{bc}	38.51^{ab}	0.221	0.004	0.005	0.002
Cystine	4.12	4.10	4.14	4.21	4.17	4.13	4.19	0.021	0.807	0.323	0.629
Glutamic acid	82.24^{a}	82.99^{ab}	83.67^{bc}	84.65^{c}	83.59^{b}	83.28^{b}	83.38^{b}	0.183	0.006	0.038	0.001
Glycine	48.77	48.70	49.14	50.11	49.85	49.47	49.42	0.172	0.232	0.079	0.117
Proline	34.34	34.71	34.78	34.72	34.77	34.59	34.66	0.058	0.449	0.388	0.089
Serine	21.96^{a}	22.10^{ab}	22.31^{b}	22.40^{b}	22.36^{b}	22.24^{ab}	22.11^{ab}	0.044	0.040	0.143	0.002
Tyrosine	14.68	14.74	14.70	14.76	14.67	14.72	14.78	0.041	0.994	0.711	0.906

All values are expressed as Mean (n = 3); Mean values in the same row with different superscripts differ significantly at 5% probability level ($p < 0.05$).

[1] Different experimental diet with graded levels of LYS (L12.3–12.3 g LYS from ingredient +0 g LYS supplementation in 1 kg diet; L14.7–12.2 g LYS from ingredient +2.5 g LYS supplementation in 1 kg diet; L17.2–12.2 g LYS from ingredient +5 g LYS supplementation in 1 kg diet; L19.6–12.1 g LYS from ingredient +7.5 g LYS supplementation in 1 kg diet; L22.3–12.3 g LYS from ingredient +10 g LYS supplementation in 1 kg diet; L24.8–12.3 g LYS from ingredient +12.5 g LYS supplementation in 1 kg diet; L27.2–12.2 g LYS from ingredient +15 g LYS supplementation in 1 kg diet).

[2] SEM, average standard error of means.

Table 6
Digestive and metabolic enzyme activities of GIFT juvenile reared in inland saline water of 10 ppt salinity and fed with graded dietary lysine levels for period of 60 days.

Enzymes		Diets[1]							SEM[2]	Contrast analysis		
		L12.3	L14.7	L17.2	L19.6	L22.3	L24.8	L27.2		*P values*		
										Overall	Linear	Quadratic
Protease[3]		3.6	4.11	3.61	4	3.67	3.96	3.79	0.091	0.693	0.826	0.706
Amylase[4]		8.32	8	9.04	8.55	8.11	8.8	8.71	0.156	0.587	0.431	0.902
Lipase[5]		6.39	5.44	7.48	6.63	7.15	7.09	6.05	0.256	0.391	0.588	0.198
AST[6]	Liver	10.95^{a}	11.75^{ab}	12.25^{bc}	13.28^{cd}	13.55^{d}	12.88^{cd}	13.23^{cd}	0.222	0.001	0.001	0.006
	Muscle	5.57^{a}	6.89^{ab}	8.21^{bc}	9.07^{c}	8.67^{c}	8.06^{bc}	7.91^{bc}	0.287	0.002	0.002	0.001
ALT[7]	Liver	7.03^{a}	8.46^{b}	9.47^{c}	10.16^{d}	9.57^{cd}	9.41^{c}	8.71^{b}	0.222	0.001	0.001	0.001
	Muscle	4.43^{a}	4.98^{ab}	6.28^{cd}	7.87^{e}	7.04^{de}	5.73^{bc}	5.23^{ab}	0.267	0.001	0.010	0.001

All values are expressed as Mean (n = 3); Mean values in the same row with different superscripts differ significantly at 5% probability level ($p < 0.05$).

[1] Different experimental diet with graded levels of LYS (L12.3–12.3 g LYS from ingredient +0 g LYS supplementation in 1 kg diet; L14.7–12.2 g LYS from ingredient +2.5 g LYS supplementation in 1 kg diet; L17.2–12.2 g LYS from ingredient +5 g LYS supplementation in 1 kg diet; L19.6–12.1 g LYS from ingredient +7.5 g LYS supplementation in 1 kg diet; L22.3–12.3 g LYS from ingredient +10 g LYS supplementation in 1 kg diet; L24.8–12.3 g LYS from ingredient +12.5 g LYS supplementation in 1 kg diet; L27.2–12.2 g LYS from ingredient +15 g LYS supplementation in 1 kg diet).

[2] SEM, average standard error of means.

[3] Protease activity is expressed as millimole of tyrosine released/mg protein/min (equivalent to 1.67^{-05} katal/mg protein or, 10^{3} U/mg protein).

[4] Amylase activity is expressed as micromole of maltose released/mg protein/min (equivalent to 1.67^{-08} katal/mg protein or, 1 U/mg protein).

[5] Lipase activity is expressed as unit/mg protein/h (equivalent to 2.78^{-4} katal/mg protein or, 1.67^{4} U/mg protein).

[6] AST, aspartate aminotransferase, the activity is expressed as nanomoles of oxaloacetate released/mg protein/min (equivalent to 1.67^{-11} katal/mg protein or, 1.00^{-03} U/mg protein).

[7] ALT, alanine aminotransferase, the activity is expressed as nanomoles of sodium pyruvate released/mg protein/min (equivalent to 1.67^{-11} katal/mg protein or, 1.00^{-03} U/mg protein).

Table 7
Serum biochemical and hematological parameters of GIFT juvenile reared in inland saline water of 10 ppt salinity and fed with graded dietary lysine levels for period of 60 days.

Parameters	Diets[1]							SEM[2]	Contrast analysis		
	L12.3	L14.7	L17.2	L19.6	L22.3	L24.8	L27.2		*P values*		
									Overall	Linear	Quadratic
Glucose (mg/dl)	88.74	86.44	85.29	83.33	84.71	82.76	85.06	0.840	0.632	0.145	0.249
Total protein (g/dl)	4.89^{a}	5.18^{b}	5.30^{b}	5.67^{d}	5.45^{c}	5.27^{b}	5.25^{b}	0.050	0.001	0.001	0.001
Albumin (g/dl)	1.18^{a}	1.29^{ab}	1.39^{bc}	1.49^{c}	1.41^{bc}	1.40^{bc}	1.38^{bc}	0.020	0.003	0.001	0.001
Globulin (g/dl)	3.71^{a}	3.89^{b}	3.91^{b}	4.21^{c}	4.04^{b}	3.88^{ab}	3.87^{ab}	0.040	0.001	0.050	0.001
A/G ratio	0.32	0.33	0.36	0.35	0.35	0.36	0.36	0.010	0.322	0.038	0.327
Hemoglobin (g/dl)	7.71^{a}	7.93^{ab}	8.10^{bc}	8.50^{de}	8.40^{cde}	8.70^{e}	8.27^{bcd}	0.080	0.001	0.001	0.004
RBC (million/cmm)	1.72	1.75	1.79	1.87	1.84	1.83	1.82	0.020	0.338	0.067	0.137
WBC (thousand/cmm)	183.14^{a}	186.93^{a}	201.40^{b}	206.37^{b}	215.93^{bc}	226.07^{c}	205.67^{b}	3.450	0.001	0.001	0.007

All values are expressed as Mean (n = 3); Mean values in the same row with different superscripts differ significantly at 5% probability level ($p < 0.05$).

[1] Different experimental diet with graded levels of LYS (L12.3–12.3 g LYS from ingredient +0 g LYS supplementation in 1 kg diet; L14.7–12.2 g LYS from ingredient +2.5 g LYS supplementation in 1 kg diet; L17.2–12.2 g LYS from ingredient +5 g LYS supplementation in 1 kg diet; L19.6–12.1 g LYS from ingredient +7.5 g LYS supplementation in 1 kg diet; L22.3–12.3 g LYS from ingredient +10 g LYS supplementation in 1 kg diet; L24.8–12.3 g LYS from ingredient +12.5 g LYS supplementation in 1 kg diet; L27.2–12.2 g LYS from ingredient +15 g LYS supplementation in 1 kg diet).

[2] SEM, average standard error of means.

tilapia reared ISW (Thomas et al., 2019). ISW has higher total alkalinity and hardness than the freshwater; however some of previous studies had suggested that euryhaline species like tilapia can tolerate this (Allan et al., 2009; Antony et al., 2021). The calcium, magnesium and potassium concentrations of each experimental unit were found to be within the desired range for GIFT culture in ISW (Singha et al., 2020).

Fish feed with ideal amino acid composition is important for achieving optimum growth (Cowey, 1994). Several studies have proven that dietary LYS inclusion at an adequate level can enhance growth performance (Santiago and Lovell, 1988; Regmi et al., 2018). In the current study, growth indices (FW, WG, WG% and SGR) were enhanced with the increasing LYS level up to 19.6 g/kg of diet and growth retardation was observed with a subsequent increasing in dietary LYS level. Growth retardation beyond the optimum level of dietary LYS might be due to the excess catabolism of LYS to generate energy with increased ammonia production causing stress, leading to reduced growth of fish (Takishita et al., 2009; Liao et al., 2015; Kotzamanis et al., 2021). Similar to the present study, Prabu et al. (2020) reported that dietary LYS at satisfactory levels could increase the growth of freshwater reared GIFT juvenile. Similarly, improvements in growth performance were also noticed in *Oreochromis niloticus* due to feeding optimal dietary LYS levels (Furuya et al., 2004; He et al., 2013; Hua et al., 2019; de Souza Romaneli et al., 2021). In the current study, feed and nutrient utilization (FCR, PER and ANPR) significantly increased in the higher LYS fed groups, whereas group provided 19.6 g LYS/kg had better nutrient utilization than the other experimental groups. Furuya et al. (2012) and Ovie and Eze (2010) observed that optimum LYS levels enhanced nutrient utilization of freshwater reared *O. niloticus*. Furthermore, Nguyen and Davis (2016) observed that dietary LYS supplementation at an adequate level could improve ANPR of *O. niloticus* juveniles.

In the present study, regression analysis revealed that the optimal dietary LYS requirement for GIFT juvenile was ranged from 19.3 to 20.7 g/kg of the diet. This dietary LYS requirement level of GIFT juvenile in ISW of 10 ppt salinity was higher than the level observed by Santiago and Lovell (1988), Bomfim et al. (2010), Furuya et al. (2012), Diogenes et al. (2016), do Nascimento et al. (2020) and Prabu et al. (2020), who found that the optimal LYS requirement for Nile tilapia fingerlings reared in freshwater could be 14.3, 18.0, 15.2, 15.6, 15.9 and 18.0 to 19.5 g/kg of diet, respectively. Whereas, compared to our result, higher dietary LYS requirements of 21.7 to 23.2 g/kg (Takishita et al., 2009) and 24.9 g/kg (Ovie and Eze, 2010) were observed in freshwater reared Nile tilapia fingerlings. Furthermore, previous studies indicate that dietary LYS requirement may be influenced by species, body size, life stage, dietary composition, salinity and other water quality parameters etc. (De Silva and Perera, 1985; Dairiki et al., 2007; He et al., 2013; Nguyen and Davis, 2016; de Souza Romaneli et al., 2021).

The changes in dietary LYS levels may not affect the survival rate of fish as noticed in the current study for GIFT juveniles and by several researchers (Prabu et al., 2020; Khalil et al., 2021; Kotzamanis et al., 2021). Similar to our result, Bomfim et al. (2010) found 100% survival when Nile tilapia fingerlings were fed with different levels of LYS.

Dietary amino acids at optimum levels can promote protein synthesis in fish by inhibiting amino acid catabolism in the presence of sufficient non-protein energy sources (Benevenga et al., 1993). The optimal dietary LYS levels support the body's protein synthesis, but inadequate or excessive LYS in feed can increase amino acid catabolism for energy production at the cost of body protein synthesis, accretion and growth (Mai et al., 2006). LYS has a role in carnitine biosynthesis (Rebouche, 1992), which has a lipotropic activity that reduces body lipid deposition (Dias et al., 2001). In the present study, higher whole-body protein and lower whole-body lipid were observed in optimum LYS fed group. However, moisture, carbohydrate and ash contents were unaffected by graded levels of dietary LYS. Adequate levels of EAAs are required for the body's protein synthesis. Therefore, high dietary LYS did not promote protein synthesis, whereas it would imbalance the normal protein metabolism (Wilson and Halver, 1986). Our results corroborated the previous data; Prabu et al. (2020) observed that increased dietary LYS levels enhanced whole-body protein and reduced the whole-body lipid level in GIFT tilapia. Similarly, many studies revealed that a LYS-deficient feed could lower body protein level in *O. niloticus* (Furuya et al., 2013; Hua et al., 2019). A decline in body lipid levels was also observed with increasing dietary LYS levels in GIFT tilapia (de Souza Romaneli et al., 2021) and *O. niloticus* (Furuya et al., 2006; Khalil et al., 2021). Several researchers obtained similar results in other species when fed with variable amounts of dietary LYS (Luo et al., 2006; Peres and Oliva-Teles, 2008; Xie et al., 2012; Ebeneezar et al., 2019; Madrid et al., 2019; Huang et al., 2021).

Generally, the EAA composition of the fish body does not alter with various water physicochemical properties and even with different sizes and species of fish (Mambrini and Kaushik, 1995). Prabu et al. (2020) stated that whole-body amino acid composition of GIFT juvenile did not change with changes in dietary composition. In the present study, the whole body EAA composition of GIFT juvenile was not altered by feeding different levels of LYS. Our results corroborated those of Michelato et al. (2016) and Prabu et al. (2020), who noticed that various dietary LYS levels could not affect the whole-body EAA composition of Nile tilapia and GIFT tilapia, respectively. Furthermore, no changes were observed in the EAA composition of *Salmo salar* fry with different LYS levels of the diet (Abboudi et al., 2006). The whole-body NEAAs such as alanine, aspartic acid, glutamic acid and serine level were raised

with the increasing dietary LYS level up to optimal (19.6 g LYS/kg); thereafter, it was either identical or decreased with increasing the dietary LYS level in the present study. These NEAAs exhibited a similar pattern to whole-body protein content. These results are in line with earlier studies by Khalil et al. (2021).

The intestinal digestive enzymes play central role in digestion of dietary nutrients and their activity is highly dependent on the composition of the feed and the availability of the corresponding substrates in the intestinal lumen (Fountoulaki et al., 2005). In the present study, intestinal digestive enzyme activities were unaffected by different levels of LYS in the diet. The experimental diets used in the present study were isonitrogenous, isolipidic and isocaloric, and probably due to this reason the activities of this enzymes remain unchanged in all the groups. Similarly, Liu et al. (2017) also failed to observe variation in digestive enzyme activities with graded levels of LYS in *Apostichopus japonicas*.

Aspartate aminotransferase and alanine aminotransferase enzymes are reallocating amino nitrogen between amino acids to synthesize NEAAs, and these amino acids further participate in body protein synthesis to heighten fish growth (Fynn-Aikins et al., 1995). In the present study, both liver and muscle AST and ALT activities displayed an increasing trend with the elevating dietary LYS levels. This trend could be correlated with higher weight gain and body protein content in the high LYS fed groups as these enzymes stimulate body protein synthesis and growth through the synthesis of newer NEAAs. Superior AST and ALT activities were also detected in *Ctenopharyngodon idella* due to increased dietary LYS levels (Li et al., 2014).

Like other animals, fish also use glucose as a major energy source, and it increases during high energy demands, which is known to indicate stress conditions (Polakof et al., 2012). In our study, no difference was found in serum glucose level with respect to graded levels of dietary LYS. Similarly, comparable serum glucose levels were also observed in Nile tilapia with different dietary LYS levels (Palupi et al., 2019; Richter et al., 2021). Serum protein level showed a positive correlation with growth and was significantly greater in the group fed with 19.6 g LYS/kg, whereas serum albumin and globulin level were higher in high LYS fed groups. These serum parameters are good indicator of fish health (Wiegertjes et al., 1996). A similar observation was also reported in GIFT juveniles fed with different amounts of dietary LYS (Prabu et al., 2020). The clear mechanism of how dietary LYS affects serum albumin, globulin, and A:G ratio is unknown and further investigation is needed to unravel the correlation between dietary LYS and serum biochemical parameters.

The graded levels of dietary LYS significantly altered the hemoglobin level and WBC count of GIFT juvenile. The higher hemoglobin and WBC counts were detected in the groups fed with 19.6, 22.3 and 24.8 g LYS/kg, whereas significantly lower hemoglobin and WBC counts were detected in the groups fed with 12.3 and 14.7 g LYS/kg. A comparable result was also observed in GIFT juvenile reared in freshwater and fed with graded levels of LYS (Prabu et al., 2020). Similarly, an improved hematological profile was noticed in Nile tilapia when the essential amino acids were fed at optimal levels (Botaro et al., 2007). The different dietary LYS levels in the present study did not alter RBC count of ISW reared GIFT juvenile. Similarly, Hisano et al. (2020) observed that feeding of LYS at various levels did not alter the blood RBC count of Nile tilapia.

5. Conclusions

Current study revealed that increment in LYS levels up to optimum requirement could significantly enhance the growth and nutrient utilization of GIFT juveniles. Feeding LYS at optimal level had an affirmative effect on whole body protein and NEAA content, whereas lipid content was negatively affected. Dietary LYS augmentation elevated the activities of aminotransferase enzymes and improved hematological and serum biochemical responses. However, body indices, survival, EAA composition of whole body, digestive enzyme activities and serum glucose level were not altered with different dietary LYS levels. Based on regression analysis, the optimal dietary LYS requirements range was found to be 19.3–20.7 g/kg of the diet for GIFT juvenile reared in ISW of 10 ppt salinity.

CRediT authorship contribution statement

Chetan K. Garg: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. **Parimal Sardar:** Conceptualization, Data curation, Supervision, Validation, Writing – original draft, Writing – review & editing. **Narottam P. Sahu:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Manas K. Maiti:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Naseemashahul Shamna:** Methodology, Formal analysis. **Tincy Varghese:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Ashutosh D. Deo:** Formal analysis, Software, Writing – review & editing. **Vungarala Harikrishna:** Software, Writing – review & editing.

Declaration of Competing Interest

None.

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