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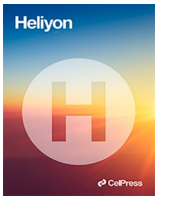

Comparative estimation of the lysine requirements in two generations of improved strain of Nile tilapia (*Oreochromis niloticus*) at the grow-out stage

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ABSTRACT

A 3 × 2 factorial experiment was conducted to investigate the effects of dietary lysine on growth performance, body indices, feed intake, feed efficiency, whole body nutrient composition and amino acid deposition in two successive generations (16th and 17th) of GIFT (*Oreochromis niloticus*). Three diets containing different levels of lysine at 1.16%, 1.56% and 2.41% were prepared for the feeding trial. Triplicate groups of fish with an initial body weight of 155 g were fed to apparent satiation for 10 weeks in a recirculating aquaculture system. Apparent digestibility coefficients (ADC) of dry matter, crude protein, crude lipids, and total carbohydrates were measured in the experimental diets. At the end of the experiment, no interactions between dietary lysine levels in diet and fish generation were observed on all parameters except for the condition factor (CF) and ADC of crude protein. However, dietary lysine level significantly affected the final weight, weight gain, thermal unit growth coefficient (TGC), protein efficiency ratio (PER) and ADC of dry matter regardless of the fish generation. Final weight, weight gain and TGC of fish were the highest in fish fed 2.41% dietary lysine in diet or 6.52% lysine in the protein. PER was the lowest in fish fed 1.16% dietary lysine. The final weight and the body's accumulation of isoleucine, phenylalanine, and alanine were significantly affected by the fish generation, with the 17th generation having the best performance. Increase growth and higher lysine requirement observed in the improved generation (17th) compared to the (16th) generation at grow out phase indicating that genetic improvement may have changed the dietary lysine requirement.

1. Introduction

Several studies have been conducted to understand the availability of amino acids in aqua feed for the development of nutritionally balanced diets, as well as the need for a balance amino acids to support growth, health, feeding efficiency and survival of Nile tilapia (*Oreochromis niloticus)* [1–5]. A balanced diet contained essential and non-essential amino acids is more important than protein [6,7].

Lysine is an essential amino acid required for fish optimal growth and its deficiency will result in lower feeding efficiency, decrease physiological performance, higher mortality, and lower weight gain and impair fish health [8,9]. Nevertheless, lysine is the most limiting amino acid when plant based ingredients is used as a primary sources in fish diet [10,11]. Therefore, lysine is often supplemented in the fish diet composition to compensate for nutrient deficiencies [12,13]. The addition of lysine to plant based fish feed is

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a common practise in aquaculture industries in order to improve fish growth and survival as well as nitrogen retention, and to reduce disease incidence and reduce fat accumulation in the body [2,6,13].

In a study with largemouth sea bass, diet supplemented with lysine was found to significantly increase growth performance, feeding efficiency, and nutrient retention by affecting the nutrient sensing signalling pathway to keep the amino acid profile in balance [14]. In addition, previous research in grass carp has shown that dietary lysine deficiency decreases growth performance, reduces antibacterial compound and lead to poor immune response and an increased inflammatory responses [15], while different study showed that it affected several important components of the IGF system and myogenesis regulators that impaired muscle growth in gilthead sea bream [16].

Tilapia is the second most produced fish species in the world after carp [17]. Tilapia production is continuously increasing globally and research is focused on improving production traits. The GIFT (Genetically Improved Farm Tilapia) was produced from selectively farmed bred tilapia (*Orechromis niloticus*) [18–20]. GIFT was genetically improved over 15 generations of selection, with a 10% increases in weight gain each generation [21]. In addition, GIFT grew 27% and 36% faster than non-GIFT [22].

Determining the lysine requirements of tilapia is necessary to produce a balanced nutritious feed. Many studies have been conducted to evaluate the optimal lysine requirement of tilapia based on growth performance, but most of these studies were conducted at the juvenile or fingerling stage [23,2,24,and11]]. Thus, there is limited information on the lysine requirement of tilapia at the grow out stage [1]. The necessity of adding lysine supplementation to the feed also resulted from the fact that fish unable to synthesize this nutrient in their bodies. However, a study have shown that supplementing a diet with lysine significantly enhance muscle development and fillet yield of adult tilapia [11]. The lysine requirement currently used in aquaculture was estimated at 1.4% of feed and 5.4% of crude protein [11], the year that the selective breeding project for GIFT began in the Philippines [25,26]. This lysine requirement is still recommended today for the cichlid *Oreochromis niloticus* [27] and for many other cichlid strains and species [28], as no study has been conducted that specifically focuses on estimating the lysine requirements of enhanced cichlid strains such as the GIFT. The objective of this study was to evaluate the lysine requirements of two GIFT's generations (16th generation and 17th generation) at the grow out stage and to investigate the interaction between dietary lysine level and fish generation on the growth performance, feed efficiency, TGC, whole body's nutrient composition and apparent digestibility coefficient of nutrient.

2. Material and methods

2.1. Ethics statement

This research was conducted in accordance with the guide and ethics for the care and use of laboratory animals National University of Malaysia (UKM) and was approved by the Research Animal Ethics Committee of the National University of Malaysia (UKMAEC), Malaysia.

Table 1
Formulation and composition of the experimental diets.

	Diet		
Ingredients (g/100g)	A:1.16% Lysine	B:1.56% Lysine	C:2.41% Lysine
[e]Distillers dried grains with soluble, DDGS	17.6	17.6	17.6
[b]Corn gluten meal	16.7	16.7	16.7
Soybean meal	24.6	24.6	24.6
Corn meal	19.3	19.3	19.3
[a]Wheat bran	7.8	7.8	7.8
[a]Canola oil	2.0	2.0	2.0
[a]Fish oil	2.9	2.9	2.9
[a]Dicalcium phosphate	2.9	2.9	2.9
[a]Mineral premix	1.0	1.0	1.0
[a]Rovimix-stay-C 25	0.1	0.1	0.1
[a]Vitamin premix	0.7	0.7	0.7
[a]Soy lecithin	0.5	0.5	0.5
[a]DL-Methionine	0.3	0.3	0.3
[c]L-Threonine	0.2	0.2	0.2
[d]L-glutamic acid	1.2	0.6	0.0
[c]L-Lysine	0.0	0.6	1.2
[f]Acid Insoluble Ash (AIA)	2.0	2.0	2.0
TOTAL (g)	**100.0**	**100.0**	**100.0**

[a] The wheat bran, fish oil, dicalcium phosphate, mineral mix, vitamin mix, soy lecithin, soya bean meal, corn meal, canola oil, rovimix-stay-C 25 ascorbyl-monophosphate and DL-methionine were obtained from Sri Purta Trading Sdn Bhd, Alor Star, Kedah, Malaysia.

[b] Corn gluten was obtained from PK-Agro industrial Products (M) Sdn Bhd, Klang, Malaysia.

[c] L-threonine and L-lysine was obtained from the local animal feed supplier Yenher Agro-products, Simpang Ampat, Penang, Malaysia.

[d] L-glutamic acid was obtained from Sigma-Aldrich (M) Sdn, Bhd, Petaling Jaya, Malaysia.

[e] Distillers dried grains with soluble (DDGS) was provided by U.S. Grains Council, Damansara Heights, Kuala Lumpur, Malaysia.

[f] Acid insoluble ash was obtained from Modern lab, Penang, Malaysia.

2.2. Experimental design and facility

The experiment was conducted using a completely randomized 3 × 2 factorial arrangement with three replicates per treatment. The first independent variable was dietary lysine, which consisted of three levels of lysine, 1.16%, 1.56% and 2.41% of diet respectively. The second independent variable was the generation or GIFT's genetic background namely, the 16th and 17th generation. The experiment was conducted for a total duration of 10 weeks, including the collection of fecal samples during the last two weeks of the experiment for nutrient digestibility analysis.

Eighteen rectangular glass aquaria of 190 L tanks in Recirculating Aquaculture System were used in this experiment. Detailed information about the rearing facility and faecal collection facility were thoroughly presented in Yossa et al. [29].

2.3. Experimental feed

Three isonitrogenous and isoenergetics experimental diets were formulated to supply required nutrients for grow out Nile tilapia (8). Three diets were formulated and supplemented with lysine at three levels (0%, 0.6% and 1.2% (as fed)) and a level of (1.2%, 0.6% and 0%) L-glutamic acid replaced the L-lysine respectively to produce a diet with a 1.16%, 1.56% and 2.41% lysine level in the diet (as is), respectively (Table 1 and Table 2). There was limited information on the lysine requirement of adult tilapia, however thee diet was formulated to provide the range within the required dietary lysine level of Nile tilapia estimated by previous study [11]. During the fecal matter collection, the experimental diets were added with 2% of acid insoluble ash (AIA) as an inert digestibility marker. Diets were prepared as dry pellets and details on the manufactured process were described in details in the previous study [29].

2.4. Experimental fish and feeding

Mixed-sexed fish of two generations of GIFT (*Oreochromis niloticus*) identified as 16th generation and 17th generation were produced by the Genetic and breeding group of WorldFish headquarter in Penang, Malaysia. Two weeks before the experiment, two batches of total 400 fish (approximate body weight of 150–155 g) were obtained and distributed equally into 18 experimental tanks according to their respective generation in order to acclimate them to the experimental conditions. During the acclimatization period fish were given commercial diet (Cargill 6123 starter 4 × 5 mm)

A day before the start of experiment, the acclimated fish were pooled into two tanks according to their respective generations. Ten fish with an initial average body weight of 154 ± 0.75 g were randomly transferred into eighteen experimental tanks, which a total of nine tanks for each generation.

Table 2
Analysed proximate composition and amino acids of the experimental diets.

	Diet		
	A:1.16% Lysine	B:1.56% Lysine	C:2.41% Lysine
Proximate composition			
Dry matter (%)	95.40	95.70	96.10
Crude protein (%)	35.53	35.74	35.59
Crude Lipid (%)	10.03	10.09	10.05
Crude Ash (%)	7.76	7.64	7.79
Crude Fiber (%)	4.11	4.18	4.01
[a]Total carbohydrate (%)	45.93	45.29	45.17
Gross energy (kcal/100 g)	423.16	421.22	419.46
Essential Amino Acids (%w/w)			
Methionine	0.95	0.84	0.94
Histidine	1.05	1.26	3.56
Isoleucine	0.10	0.10	0.10
Leucine	2.63	2.52	2.41
Lysine	1.16	1.58	2.41
Phenylalanine	0.73	0.84	0.73
Threonine	6.50	6.48	6.49
Valine	0.63	0.63	0.62
Arginine	1.05	1.05	1.04
Tyrosine	1.47	1.89	1.15
Non-essential amino acid (%w/w)			
Proline	2.11	2.00	1.88
Glycine	1.16	1.16	1.26
Glutamic Acid	4.93	5.02	4.47
Aspartic Acid	2.63	2.73	1.78
Alanine	2.11	2.10	1.57
Serine	1.68	1.58	1.36

[a] Total carbohydrate content calculated as: total carbohydrate (% dry matter) = 100 – ash (%Dry matter) – crude protein (% Dry matter) – lipid (% Dry matter).

Fish were hand fed to apparent satiation three times daily (8am, 12pm and 4pm) for 10 weeks for the whole experiment duration, except during the biweekly sampling and final sampling. The feeding duration was determined by the loss of feeding activity after fish were fed at least three independence feeding episodes over 1 h each feeding.

2.5. Data and sample collection

During the initial stocking, fish were measured individually for their body weight, total length and standard length before transferred into the experimental tank. Ten fish of each generation were randomly selected from the remaining stock to determine initial whole body's proximate composition and amino acids. Then, another ten fish of each generation were dissected to determine the gonad and liver weight for the calculation of gonadosomatic index (HSI) and hepatosomatic index (GSI), respectively. After two weeks and every consecutive two weeks, fish were weighed in bulk and counted in order to record their biweekly growth and survival rate. Before the measurement, fish were anaesthetized with 40–100 mg/L clove oil to minimize stress.

At the end of the experiment, fish from each tank were counted and individual fish were weighed and total length and standard length were measured. Four to five fish from each tank were randomly dissected to determine their liver and gonad weight for HSI and GSI respectively. Then the liver and gonads were reinserted into the respective fish body. The liver and gonad were all inserted into the fish body and immediately after that the whole body of fish were dried for further processing and then were frozen before the analysis of whole body nutrients and amino acid composition. During the initial and intermittent sampling, fish were anaesthetized with clove oil (40–100 mg/L) before weighing and during the last sampling fish were euthanized with an overdose of clove oil (400–500 mg/L) before dissection. Fish carcasses were immediately stored at −20 °C and then dried at 60 °C using a dryer (VENTICELL, model LSIS-B2V/VC 404, MMM Medcenter Einrichtungen GmbH; Germany) for 12 h. Then the samples were grinded using a dry mill (Panasonic, MX-GM 1011) before stored at −20 °C for subsequent analysis.

During the latter phase, the daily collection of faecal material started at the end of the day, following the last feeding and the cleaning of each aquarium to remove any feed or faecal particles, and ended on the following morning prior to the first feeding of the day. The collection of faecal material from each aquarium was done using a faeces settling unit (ECO-TRAP 110 Waste solid Removal system, PENTAIR, USA) to which a bottle was attached. The bottle received the faecal matter coming from each tank, and was maintained on ice in order to prevent the nutrient degradation. Detailed information about faecal collection was described in previous digestibility study [29].

Feed given to fish were recorded daily for each tank. Uneaten feed from each tank was collected, put in a labelled plastic bag and kept in the freezer (−20 °C). After the experiment completed, the uneaten feed from the same tank were pooled and dried for 12 h at 60 °C and stored in the freezer (−20 °C) prior to analysis.

2.6. Data analysis

Growth parameters were determined using the following formulae:

$$Weight\ gain\ (g) = Mean\ final\ body\ weight\ (g) - Mean\ initial\ body\ weight\ (g)$$

$$Feed\ intake\ (DMg\ /\ fish) = \frac{Total\ Feed\ given\quad (DM, g) - Total\ uneaten\ feed\ (DM, g)}{Fish\ number}$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Total\ feed\ intake\ (g)}{Total\ fish\ weight\ gain\ (g)}$$

$$Hepatosomatic\ Index\ (HSI) = \frac{Liver\ weight\ (g)}{Fish\ weight\ (g)} x\ 100$$

$$Gonadosomatic\ index\ (GSI) = \frac{Gonad\ weight\ (g)}{Fish\ weight(g)}\ x\ 100$$

$$Protein\ efficiency\ ratio\ (PER) = \frac{Weight\ gain\ (g)}{Total\ protein\ intake\ (g)}$$

$$\text{Condition factor (CF)} = \text{weight of fish} \ / \ (\text{length of fish}) \times 100$$

$$Thermal\ growth\ coefficiant\ (TGC)\ \left(g1/3/_{\circ} CxDay = 1000\ \text{x}\ \left[\left(\text{FBW}^{1/3} - \text{IBW}^{1/3} \right) \sum (T\ xD) \right] \right.$$

where $\sum$ (T x D) = sum of temperature (°C) recorded x days.

FBW= Final body Weight.

IBW= Initial body weight

Table 3
Growth performance, feed utilization, survival rate and TGC of 16th and 17th generation of GIFT fed diet containing three lysine levels for 10 weeks.

Dietary Lysine	Fish generation	Initial weigh(g)	Final weight (g)	Weight gain (g)	FI (gDM fish-1) day-1)	FCR	PER	Survival (%)	TGC (gl/3/C° day)
1.16%	17th generation	155.18 ± 0.47	312.96 ± 14.62	152.54 ± 20.25	382.04 ± 51.95	2.53 ± 0.25	1.14 ± 0.11	70.00 ± 0.15	1.13 ± 0.11
1.56%	17th generation	154.59 ± 0.23	296.66 ± 12.91	138.17 ± 13.59	301.06 ± 26.58	2.25 ± 0.37	1.29 ± 0.25	76.67 ± 0.12	1.04 ± 0.08
2.41%	17th generation	156.19 ± 0.60	353.48 ± 13.99	197.72 ± 19.57	376.73 ± 19.06	2.00 ± 0.07	1.41 ± 0.01	83.33 ± 0.03	1.38 ± 0.10
1.16%	16th generation	154.53 ± 0.89	280.11 ± 7.52	125.58 ± 4.40	268.59 ± 14.45	2.15 ± 0.19	1.33 ± 0.11	83.33 ± 0.04	0.96 ± 0.02
1.56%	16th generation	153.96 ± 0.52	286.24 ± 10.15	132.07 ± 11.77	304.73 ± 50.79	2.31 ± 0.33	1.23 ± 0.06	76.67 ± 0.06	1.0 ± 0.07
2.41%	16th generation	154.85 ± 0.72	324.84 ± 13.32	174.95 ± 19.57	395.33 ± 91.92	2.21 ± 0.25	1.26 ± 0.16	76.67 ± 0.06	1.26 ± 0.13
Dietary lysine									
1.16%		154.85 ± 0.47	295.44 ± 8.19[a]	139.06 ± 11.05[a]	325.31 ± 35.00	2.31 ± 0.16	1.23 ± 0.08	75.00 ± 18.4	1.04 ± 0.06[a]
1.56%		154.28 ± 0.29	291.45 ± 8.16[a]	135.11 ± 20.97[a]	302.89 ± 25.65	2.28 ± 0.22	1.26 ± 0.14	76.67 ± 15.7	1.02 ± 0.05[a]
2.41%		155.52 ± 0.51	340.04 ± 9.84[b]	186.33 ± 14.49[b]	386.02 ± 42.19	2.10 ± 0.12	1.33 ± 0.84	80.00 ± 1.40	1.31 ± 0.08[b]
Fish Generation									
17th generation		155.32 ± 0.98	322.65 ± 8.43[b]	162.80 ± 12.72	353.27 ± 22.06	2.22 0.13	1.28 ± 0.08	77.7 ± 18.4	1.07 ± 0.06
16th generation		154.48 ± 0.38	296.82 ± 6.44[a]	144.20 ± 10.86	322.88 ± 35.94	2.25 0.15	1.27 ± 0.09	76.67 ± 13.8	1.18 ± 0.07
Two-way ANOVA									
Dietary lysine		NS	***	**	NS	NS	NS	NS	*
Fish generation		NS	*	NS	NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS	NS	NS	NS

FI: Feed intake; FCR: Feed conversion ratio; PER: Protein efficiency ratio; TGC: thermal growth coefficient.
Mean with different superscript letters indicate significant differences ($p < 0.05$).
Values presented as means and pooled standard error of the mean (SEM).Two-way ANOVA: NS: non-significant ($P > 0.05$); *$P < 0.05$ **$P < 0.005$, ***$P < 0.0005$.

$$Survival\ rate\ (\%) = \frac{Final\ Number\ of\ fish}{Initial\ number\ of\ stocking}\ x\ 100\%$$

The apparent digestibility coefficients (ADCs) of the nutrients in the diet were calculated as follows [8].

$$ADC\ of\ nutrient\ (\%) = 1 - \left(\frac{\%AIA\ in\ diet(DM)}{\%AIA\ in\ feces(DM)}\right) x \left(\frac{\%Nutrient\ in\ feces(DM}{\%Nutrient\ in\ diet(DM}\right)$$

where AIA is an acid insoluble ash, the digestibility marker.

2.7. Chemical analysis (proximate analysis and amino acid of fish)

The experimental fish carcass were analysed for proximate analysis (dry matter, crude protein, crude lipid, crude ash, crude fiber, gross energy) and amino acids profile. Similar analyses were conducted on fecal material and feed, with addition to the acid insoluble ash (AIA) analysis. The method used to analyses those parameters followed the method; MS ISO 6496:2003 [30], ISO 1871:2009 [31], Method 3:0 [32], ISO 5984:2002 [33], Method 9:0 [34], calculation (based on Method of Analysis for Nutrition Labelling, AOAC, 1993, page 106 & 5) [35] and AOAC 994.12 [36], respectively, as described in Ref. [29].

2.8. Statistical analysis

All calculation was performed using R studio software Version 4.0.2 (2020-06-22). All variables were fit into the linear regression model using a *lm* function of the package in R. All growth parameters, fish whole body proximate and amino acids, and ADC variables were statistically analysed with two-way ANOVA to determine the effects of dietary lysine and fish generation as well as their interaction. The assumption of the homogeneity of variances was verified by Levene's test, and the normality of residuals was assessed by the Shapiro–Wilk test. When results were found to be significant ($p < 0.05$), the data were further subjected to a comparison of means using the Tukey HSD test. The Tukey post hoc test was performed using Agricolae package to explore the differences between groups whenever interaction or main effect was significant ($P < 0.05$).

3. Result

3.1. Growth performance, feed intake, feed efficiency and survival

No significant interaction was found between dietary lysine and fish generation on growth performance, feed intake, feed efficiency and fish survival and TGC (Table 3; $P > 0.05$). Dietary lysine has a significant main effects on final weight, weight gain and TGC ($P < 0.05$) but did not significantly affected feed intake, PER, FCR and survival ($P > 0.05$). Meanwhile, fish generation showed a significant main effects only on the final weight. Among the three dietary lysine levels, the highest final body weight and weight gain was observed in tilapia fed 2.41% dietary lysine level which were 340.04 g and 186.33 g respectively. The lowest final body weight was observed in fish fed 1.56% dietary lysine; however, it was not significantly different with fish fed with 1.16% dietary lysine. Fish fed

Table 4
Condition Factors (CF), Hepatosomatic index (HSI) and Gonadosomatic index (GSI) of 16th and 17th generation of GIFT fed diets containing three lysine levels for 10 weeks.

		CF	HSI	GSI
Dietary Lysine	**Fish generation**			
1.16%	17th generation	1.52 ± 0.02^{ab}	1.67 ± 0.33	1.61 ± 0.12
1.56%	17th generation	1.47 ± 0.02^{a}	1.76 ± 0.46	1.99 ± 0.23
2.41%	17th generation	1.58 ± 0.03^{b}	0.99 ± 0.25	1.88 ± 0.15
1.16%	16th generation	1.55 ± 0.02^{ab}	1.81 ± 0.34	2.25 ± 0.17
1.56%	16th generation	1.54 ± 0.02^{ab}	1.64 ± 0.37	2.57 ± 0.81
2.41%	16th generation	1.55 ± 0.02^{ab}	2.13 ± 0.29	1.75 ± 0.17
Dietary lysine				
1.16%		1.52 ± 0.02	1.75 ± 0.23	1.95 ± 0.12
1.56%		1.53 ± 0.02	1.74 ± 0.30	2.23 ± 0.36
2.41%		1.55 ± 0.01	1.57 ± 0.21	1.82 ± 0.11
Fish Generation				
17th generation		1.528 ± 0.01	1.88 ± 0.19	1.82 ± 0.21
16th generation		1.547 ± 0.01	1.49 ± 0.21	2.14 ± 0.21
Two-way ANOVA				
Dietary lysine		NS	NS	NS
Fish generation		NS	NS	NS
Interaction		0.00**	NS	NS

Mean with different superscript letters indicate significant differences ($p < 0.05$).
Values presented as means and pooled standard error of the mean (SEM).
Two-way ANOVA: NS: non-significant ($P > 0.05$); **$P < 0.005$.

with 2.41% dietary lysine level also showed the significant highest in the TGC value. Meanwhile, between the two generations, the 17th generation showed the significant highest final body weight compared to the 16th generation.

3.2. Condition factor, hepatosomatic index and gonadosomatic index

There was a significant interaction between dietary lysine level and fish generation on the CF (Table 4; $P < 0.05$) but not for HSI and GSI (Table 4; $P > 0.05$). The lowest mean of CF value was observed in the 17th generation fed with 1.56% lysine and the highest value was from 17th generation fed 2.41% lysine in diet. Meanwhile no significant differences in CF were observed between fish of 17th generation fed 1.16% lysine with 16th generation fed 1.16%, 1.56% and 2.41% respectively. In addition, there was no significant different in CF, HSI and GSI between the dietary lysine treatment or fish generation respectively.

3.3. Chemical analysis (fish proximate analysis and amino acid composition)

There was no significant interactions between dietary lysine and fish generation on the dry matter crude protein, crude lipid, crude ash, total carbohydrate and gross energy in the whole body of fish (Table 5; $P > 0.05$).

Similarly, there was also no significant interaction between dietary lysine and fish generation (Table 6; $P > 0.05$) on the amino acids composition in the whole body of fish. Fish generation showed a significant effect on the deposition of isoleucine, phenylalanine and alanine. 17th generation had the highest phenylalanine deposition meanwhile the 16th generation had the highest alanine and isoleucine ($p < 0.05$). Meanwhile the other amino acids depositions were not significantly affected by dietary lysine or fish generation ($p > 0.05$).

3.4. Apparent digestibility coefficient (ADC)

There was no significant interaction between dietary lysine and fish generation on ADC of dry matter, crude lipid and total carbohydrate but there was a significant interaction (Table 7 $P > 0.05$) on ADC of crude protein (Table 7 $P > 0.05$). There was a significant

Table 5
Proximate analysis of whole body of 16th and 17th generation GIFT fed diets containing three lysine levels for 10 weeks.

		Dry matter (%)	Crude protein (%)	Crude Lipid (%)	Crude Ash (%)	**Total carbohydrate (%)	Gross energy (kcal/ 100 g)
Dietary Lysine	**Fish generation**						
1.16%	17th generation	25.9 ± 2.04	57.4 ± 0.85	17.43 ± 0.99	15.83 ± 0.56	9.32 ± 0.59	468.62 ± 4.06
1.56%	17th generation	29.3 ± 0.10	55.24 ± 0.87	16.98 ± 0.41	17.88 ± 0.92	9.87 ± 0.88	454.09 ± 8.14
2.41%	17th generation	24.23 ± 4.12	54.43 ± 0.68	20.62 ± 1.68	15.7 ± 0.83	9.24 ± 0.95	486.44 ± 12.49
1.16%	16th generation	30.21 ± 0.75	56.5 ± 1.00	17.46 ± 1.05	15.64 ± 0.53	10.39 ± 0.47	467.89 ± 4.86
1.56%	16th generation	28.86 ± 0.56	56.67 ± 1.21	19.07 ± 1.47	16.74 ± 0.24	7.50 ± 0.33	474.47 ± 6.27
2.41%	16th generation	29.86 ± 3.05	55.65 ± 0.64	19.49 ± 0.76	15.65 ± 0.01	9.19 ± 0.30	477.68 ± 7.63
Dietary lysine							
1.16%		28.06 ± 1.36	56.95 ± 0.69	17.45 ± 0.64	15.73 ± 0.35	9.86 ± 0.41	468.25 ± 2.83
1.56%		29.12 ± 0.72	55.96 ± 0.74	18.03 ± 0.83	17.31 ± 0.49	8.69 ± 0.67	464.28 ± 6.99
2.41%		27.05 ± 2.61	55.04 ± 0.50	20.06 ± 0.86	15.68 ± 0.58	9.21 ± 0.44	482.06 ± 6.83
Fish Generation							
17th generation		26.51 ± 1.53	55.69 ± 0.63	18.35 ± 0.81	16.01 ± 0.38	9.48 ± 1.27	473.34 ± 3.88
16th generation		29.64 ± 0.94	56.27 ± 0.51	18.67 ± 0.64	16.47 ± 0.52	9.03 ± 0.414	469.72 ± 6.46
Two-way ANOVA							
Dietary lysine		NS	NS	NS	NS	NS	NS
Fish generation		NS	NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS	NS

Total carbohydrate content calculated as: total carbohydrate (% Dry matter) = 100 – ash (%Dry matter) – crude protein (% Dry matter) – lipid (% Dry matter).
Values presented as means and pooled standard error of the mean (SEM).
Mean with different superscript letters indicate significant differences ($p < 0.05$). Two-way ANOVA: NS: non-significant ($P > 0.05$).

Table 6
Analysed essential and non-essential amino acid composition in whole body of 16th and 17th generation of GIFT fed diet containing three lysine levels for 10 weeks.

		Essential Amino Acids								
		Lysine	Methionine	Threonine	Arginine	Histidine	Isoleucine	Leucine	Phenylalanine	Valine
Dietary lysine	**Fish Generations**									
1.16%	17th generation	2.43 ± 0.16	1.06 ± 0.72	5.07 ± 1.11	1.95 ± 0.08	0.63 ± 0.14	0.18 ± 0.07	3.16 ± 0.52	1.45 ± 0.35	1.34 ± 0.14
1.56%	17th generation	3.38 ± 0.92	1.28 ± 1.51	4.81 ± 1.00	2.52 ± 0.42	0.99 ± 0.42	0.28 ± 0.12	3.20 ± 0.47	2.19 ± 0.86	1.36 ± 0.30
2.41%	17th generation	2.09 ± 0.67	0.95 ± 0.15	4.60 ± 0.72	1.84 ± 0.27	0.60 ± 0.18	0.17 ± 0.05	4.01 ± 0.99	1.68 ± 0.52	1.73 ± 0.77
1.16%	16th generation	2.79 ± 1.55	1.32 ± 0.13	6.30 ± 1.23	2.61 ± 0.29	1.03 ± 0.33	0.43 ± 0.09	2.76 ± 0.83	3.54 ± 0.79	1.03 ± 0.14
1.56%	16th generation	3.32 ± 0.13	1.25 ± 0.04	4.01 ± 0.39	2.51 ± 0.23	1.19 ± 0.45	0.36 ± 0.15	3.52 ± 0.60	4.38 ± 0.361	1.50 ± 0.05
2.41%	16th generation	3.00 ± 1.16	1.22 ± 0.17	4.53 ± 1.26	2.48 ± 0.48	1.59 ± 0.53	0.45 ± 0.09	4.00 ± 1.17	2.61 ± 0.47	2.03 ± 0.76
Dietary lysine										
1.16%		2.61 ± 0.70	1.19 ± 0.09	5.68 ± 0.79	0.28 ± 0.20	0.82 ± 0.19	0.30 ± 0.06	2.96 ± 0.44	2.50 ± 0.60	1.18 ± 0.11
1.56%		3.35 ± 0.41	1.26 ± 0.07	4.41 ± 0.49	2.52 ± 0.22	1.09 ± 0.28	0.32 ± 0.08	3.36 ± 0.35	3.29 ± 0.64	1.48 ± 0.14
2.41%		2.54 ± 0.63	1.08 ± 0.12	4.57 ± 0.65	2.16 ± 2.99	1.09 ± 0.33	0.31 ± 0.07	4.00 ± 0.69	2.14 ± 0.37	1.88 ± 0.49
Fish Generations										
17th generation		2.63 ± 0.38	1.1 ± 0.06	4.82 ± 0.48	2.10 ± 0.18	0.73 ± 0.15	0.21 ± 0.04[a]	3.45 ± 0 .37	3.51 ± 0.38[b]	1.47 ± 0.25
16th generation		3.03 ± 0.56	1.26 ± 0.06	4.95 ± 0.62	2.53 ± 0.17	1.27 ± 0.24	0.41 ± 0.05[b]	3.42 ± 0.48	1.77 ± 0.32[a]	1.55 ± 0.26
Two-way ANOVA										
Dietary lysine		NS	NS	NS	NS	NS	NS	NS	NS	NS
Fish generation		NS	NS	NS	NS	NS	*	NS	**	NS
Interaction		NS	NS	NS	NS	NS	NS	NS	NS	NS

		Non-essential amino acid (%W/W)					
		Proline	Glycine	Glutamic Acid	Aspartic Acid	Alanine	Serine
Dietary lysine	**Fish Generations**						
1.16%	17th generation	2.14 ± 0.18	3.31 ± 0.08	4.24 ± 0.21	3.23 ± 0.35	2.92 ± 0.10	1.26 ± 0.10
1.56%	17th generation	3.65 ± 0.81	4.43 ± 0.33	4.62 ± 0.07	3.65 ± 0.67	3.86 ± 0.46	1.60 ± 0.15
2.41%	17th generation	0.92 ± 0.34	3.47 ± 0.40	3.85 ± 0.33	2.88 ± 0.18	3.67 ± 0.82	1.16 ± 0.20
1.16%	16th generation	1.50 ± 0.78	4.00 ± 0.44	3.85 ± 0.35	2.72 ± 0.61	4.14 ± 0.12	1.61 ± 0.15
1.56%	16th generation	2.42 ± 1.06	4.26 ± 0.38	3.62 ± 0.13	5.08 ± 0.90	4.95 ± 0.63	1.72 ± 0.14
2.41%	16th generation	1.76 ± 0.73	3.82 ± 0.53	3.57 ± 0.13	3.66 ± 0.81	3.85 ± 0.27	1.53 ± 0.22
Dietary lysine							
1.16%		1.83 ± 0.38	3.65 ± 0.25	1.04 ± 0.20	2.98 ± 0.33	3.53 ± 0.28	1.42 ± 0.11
1.56%		3.04 ± 0.66	4.37 ± 1.23	4.12 ± 0.53	4.37 ± 0.59	4.40 ± 0.42	1.66 ± 0.09
2.41%		1.34 ± 0.40	3.6 ± 0.31	3.71 ± 0.17	3.27 ± 0.41	3.76 ± 0.38	1.35 ± 0.16
Fish Generations							
17th generation		2.24 ± 0.47	4.02 ± 0.23	4.24 ± 0.34	3.26 ± 0.24	3.48 ± 0.26[a]	1.33 ± 0.10
16th generation		1.90 ± 0.45	3.75 ± 0.24	3.60 ± 0.12	3.82 ± 0.52	4.31 ± 0.26[b]	1.62 ± 0.09
Two-way ANOVA							
Dietary lysine		NS	NS	NS	NS	NS	NS
Fish generation		NS	NS	NS	NS	**	NS
Interaction		NS	NS	NS	NS	NS	NS

Mean with different superscript letters indicate significant differences ($p < 0.05$).
Values presented as means and pooled standard error of the mean (SEM). Two-way ANOVA: NS: non-significant ($P > 0.05$); *$P < 0.05$; **$P < 0.005$.

Table 7
Apparent Digestibility Coefficient of nutrient in diet of 16th and 17th generation of GIFT containing different level of lysine.

Dietary lysine	Fish generation	Dry matter (%)	Crude Protein (%)	Crude Lipid (%)	Total Carbohydrate (%)
1.16%	17th generation	93.58 ± 1.37	61.80 ± 1.25	65.92 ± 0.28	47.22 ± 2.25
1.56%	17th generation	94.71 ± 1.10	63.50 ± 1.44	64.92 ± 3.37	53.98 ± 0.26
2.41%	17th generation	91.26 ± 0.54	51.07 ± 0.91	63.38 ± 2.28	38.70 ± 3.08
1.16%	16th generation	94.08 ± 1.42	54.72 ± 4.07	60.96 ± 2.08	44.21 ± 4.20
1.56%	16th generation	95.22 ± 0.59	52.55 ± 1.94	61.12 ± 4.60	44.27 ± 5.28
2.41%	16th generation	92.06 ± 2.07	55.19 ± 4.38	65.02 ± 2.60	45.54 ± 5.28
Dietary lysine					
1.16%		93.83 ± 0.89 [ab]	58.26 ± 2.47	63.44 ± 1.98	45.72 ± 2.23
1.56%		94.96 ± 0.51[b]	58.02 ± 2.67	63.02 ± 2.69	49.13 ± 3.21
2.41%		91.66 ± 0.97[a]	53.13 ± 2.20	64.20 ± 1.59	42.12 ± 3.61
Fish generation					
17th generation		93.18 ± 0.73	58.79 ± 1.86	64.74 ± 1.51	44.67 ± 2.74
16th generation		93.79 ± 0.86	54.15 ± 1.86	62.37 ± 1.77	46.63 ± 2.47
Two-way ANOVA					
Dietary lysine		*	NS	NS	NS
Fish generation		NS	NS	NS	NS
Interaction		0 NS	*	NS	NS

Mean with different superscript letters indicate significant differences ($p < 0.05$).
Values presented as means and pooled standard error of the mean (SEM).
Two-way ANOVA: NS: non-significant ($P > 0.05$); *$P < 0.05$.

interaction between dietary lysine and fish generation on the ADC of crude protein but no significant different was found between the treatments ($P > 0.05$). Although no significant interaction between the main factors but dietary lysine was significantly affected ADC of dry matter ($p < 0.05$). The ADC dry matter was the lowest in the fish fed 2.41% dietary lysine and the highest in the fish fed 1.56% dietary lysine ($P < 0.05$) but both were not significantly different with fish fed 1.16% dietary lysine. Meanwhile, fish generation did not significantly affect the ADCs of dry matter, crude protein, crude lipid and total carbohydrate ($p > 0.05$).

4. Discussion

The present study found that there was no significant interaction between dietary lysine and fish generation on the growth performance, feed intake and efficiency, HSI, GSI, whole body nutrient composition and ADC of nutrients of GIFT. The interaction was only found in the CF and ADC of Protein.

Regardless of generations, lysine requirement for the grow out GIFT in this study was estimated to be 2.4% of the diet, and when expressed as a ratio to dietary protein, the dietary lysine requirement estimated in this work was 6.5% of dietary protein, based on the final body weight and weight gain of the fish. The lysine requirement for the growth of grow out GIFT estimated in this study was comparable to and higher than the values reported in other the previous study reported by Ref. [1] who estimated lysine requirement of 5.87% dietary protein for adult Nile Tilapia weighing 160 g cultured in concrete tank. In related to this, a higher lysine requirement for fish can be attributed to the life stage of the fish, fish species, genetic variation, rearing condition and amino acid pattern in the diet [37,38]. In addition, the 17th generation showed a better final weight than the 16th generation suggested that genetic improvement is effective to improve growth of each new generation of the GIFT [19,25].

Lower weight gain and final weight were observed in the fish fed with lower lysine levels and similar treatment also resulted in lower feed intake and feed efficiency. This trend was similar with the observation in which feed intake was highest on feed with the highest lysine content and decreased linearly as the dietary lysine content was reduced [12]. However, there is no consensus on whether the higher growth rates were due to their increased ability to consume feed or to a greater ability to convert nutrients into body mass. Despite, the increased tilapia growth in this study suggested that adult tilapia was able to efficiently utilize dietary with a lysine supplemented in the formulated feed.

FCR value for the fish in this study was greater than 2.0 and this was estimated using feed from non-extruded pelleted, whilst contrary with FCR for tilapia raised in tanks typically range from 1.2 to 2.0 for extruded [39]. In relation to this, the pellet manufacturing method could contributed to the higher FCR, in addition it was also observed that daily FCR was higher in older animals compared to young animals due to the slower growth rate during the grow out stage. Dietary lysine had a significant effect on TGC, with the highest value observed when fish were fed a diet containing 2.41% lysine. This is in consistent with previous finding that as the lysine level in the diet increased, the TGC of rainbow trout was also increased [40].

The HSI value and SGI value both continue to rise during the development of gonadal maturity except for when the fish spawn. During the spawning GSI value reaches the upper range limit and the HSI value decreases. Generally, the somatic gonadal index value and the hepatosomatic index value are related. The fact that the 16th generation had a lower HSI value and a higher GSI value than the 17th generation indicated that the 16th generation in this study matured earlier than the 17th generation. HSI and GSI values also reflect visceral fat accumulation caused by unbalanced diets [12], whereas CF is a parameter that indicates a fish's health based on its weight and length [41]. [42] Reported that the condition factor (CF) and hepatosomatic index (HSI) were significantly affected by

dietary lysine levels in juvenile fish sucker (*Myxocyprinus asiaticus)*. However this trend was not observed in the present study. In contrast, dietary lysine and fish generation had no effect on CF, HSI, or GSI, indicating that these factors did not affect GIFT body indices. The CF value obtained in this study ranged between 1.47 and 1.58, indicating that the fish was healthy and in a good condition, as a condition factor greater than 1.0 denotes healthy fish and isometric growth [43].

In the present study, there was no significant interaction between dietary lysine and fish generation on the dry matter, crude lipid, crude ash, total carbohydrate and gross energy, and no significant different was found between dietary lysine and fish generation on all the parameters. The non-significant interaction and differences indicated that lysine supplementation did not affect the nutrients storing in the whole fish body. The reason for this could be due to the all experimental diets had a similar protein content, as it was found that dietary lysine supplement did not affect the whole body of juvenile GIFT when fed similar protein content in the diet [12, 23]. In contrast [44], suggested that fish supplemented with balanced amino acids and same digestible protein content in the diet showed significant increases in the percentage of protein and decreases in the percentage of lipids in the body tissue. Similarly [45], found that Nile tilapia fed a diet deficient in lysine displayed a lower protein content and a higher body fat percentage however the trend was not observed in this study.

This study discovered that fish generation had a significant effect on isoleucine, phenylalanine, and alanine deposition in the tissue body. The highest deposition of phenylalanine in generation 17th and the highest deposition of isoleucine and alanine in generation 16th is difficult to explain, but suggested a greater potential of those nutrients to be utilized by the respective generations of GIFT. There was no significant effect of dietary lysine on all other amino acids in fish whole body. This finding was consistent with previous research on the effect of acid amino in whole-body amino acid profiles on different species [23, 40, and 41]]. In contrast, it was discovered that increasing dietary lysine levels affected whole body lysine in Tilapia [12]. Yet, there is a lack of information on the effect of dietary lysine on the retention of amino acids in the whole body of grow out tilapia.

The significant interaction between dietary lysine and fish generation on ADC of crude protein indicated the genetic improvement plays a role in the utilization of lysine in protein digestion, and this should be further investigated. The ADC value obtained from this study was lower than the suggested range of 75%–97% [46,47]. The reasons could be because of the method of diet manufacturing, ingredient type and faeces collection method. Generally, ingredient from plant is normally less digestible than animal based diets for tilapia. Faecal material collection method could also lead to the underestimated of ADC value as it was widely known that the method of collection is one of the factors affecting digestibility estimation [48]. In this study, we used a swirl separator to collect the faecal material which then settled in the collecting bottle. Although careful precautious was made to avoid the degradation of the faecal matter, and thanks to the use of ice but the leaching of nutrients into the water still could happen.

ADC of dry matter were the lowest when fish were fed the highest dietary lysine level. There were limited information limited information on how dietary lysine affects ADC of dry matter but a study by Ref. [49] suggested that a lower value of ADC nutrients might be due to the high fibre content in the diet which decreased the digestibility. Nutritional values of a diet will depend on how efficient its nutrient can be digested, and since there may be interactions amongst the ingredients, the additivity of each ingredient's digestibility should be addressed.

5. Conclusion

The optimal dietary lysine requirement for grow out GIFT without considering the genetic improvement was estimated at 2.41% of diet or 6.52% of dietary protein, which was higher than estimated for adult tilapia. Furthermore, when comparing the two generations' final weight, weight gain, and lysine requirement, the 17th generation outperformed the 16th generation. Thus, the greater performance suggested that selective breeding program to produce GIFT to improve tilapia production has successful and resulted in higher dietary lysine requirement. Therefore, in the future in order to ensure that the fish can satisfy new dietary requirements for optimal growth and health, it is recommended that the important nutrients requirement for new GIFT generation be constantly investigated and revised.

Author contribution statement

Nurulhuda Ahmad Fatan; Kamini Sivajothy: Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Rodrigue Yossa: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

The data produced and used in this study are available on request from the corresponding author.

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.

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