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Salinity levels affect the lysine nutrient requirements and nutrient metabolism of juvenile genetically improved farmed tilapia (*Oreochromis niloticus*)

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Abstract

This 62-d research aimed to evaluate the effects of dietary lysine levels (DLL) and salinity on growth performance and nutrition metabolism of genetically improved farmed tilapia (GIFT) juveniles (*Oreochromis niloticus*). Six diets with lysine supplementation (1·34, 1·70, 2·03, 2·41, 2·72 and 3·04 % of DM) were formulated under different cultured salinities in a two-factorial design. The results indicated that supplemental lysine improved the specific growth rate (SGR) and weight gain (WG) and decreased the feed conversion ratio (FCR). Meanwhile, the fish had higher SGR and WG and lower FCR at 8 ‰ salinity. Except for moisture, the whole-body protein, lipid and ash content of GIFT were increased by 8 ‰ salinity, which showed that DLL (1·34 %) increased the whole-body fat content and DLL (2·41 %) increased whole-body protein content. Appropriate DLL up-regulated mRNA levels of protein metabolism-related genes such as target of rapamycin, 4EBP-1 and S6 kinase 1. However, 0 ‰ salinity reduced these protein metabolism-related genes mRNA levels, while proper DLL could improve glycolysis and gluconeogenesis mRNA levels but decrease lipogenesis-related genes mRNA levels in liver. 0 ‰ salinity improved GLUT2, glucokinase and G6 Pase mRNA levels; however, sterol regulatory element-binding protein 1 and fatty acid synthase mRNA levels were higher at 8 ‰ salinity. Moreover, 8 ‰ salinity also increased plasma total protein and cholesterol levels and decreased glucose levels. These results indicated that the recommended range of lysine requirement under different salinity was 2·03–2·20 % (0 ‰) and 2·20–2·41 % (8 ‰) and 8 ‰ salinity resulted in higher lysine requirements due to changes in the related nutrient metabolism, which might provide useful information for designing more effective feed formulations for GIFT cultured in different salinity environment.

Keywords: **Genetically improved farmed tilapia: Lysine requirements: Salinity: Nutrient metabolism**

Tilapia is a farmed species worldwide whose production reached 103·0 million tons in 2018[1]. Genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) is a commercially vital farmed tilapia species due to its high adaptability and rapid growth rate[2]. However, the lack of freshwater resources and improved breeding costs limit the further development of the tilapia aquaculture industry. Globally, rising temperature causes sea level to rise, which intensifies salinisation in coastal areas. The freshwater shortage is an inevitable burden, and more than 1·2 million ha of land have been lost yearly to salinisation[3]. It has been estimated that saline-alkaline water covers approximately 45·87 million ha in China alone, and salinity mainly ranged between 6 and 10 ‰[4,5]. Fortunately, saline-alkaline could be utilised to produce food through aquaculture[6]. Song *et al.*[7] found no significant negative effects of saline-alkaline water on the health of Nile tilapia and Qiang *et al.*[8] recommended GIFT tilapia juveniles be cultured at 7·8 ‰. Accordingly, as a new euryhaline species, GIFT has more potential as a new alternative for aquaculture in inland and low-salinity waters, which is meaningful for alleviating the pressure of freshwater aquaculture and meeting the increasing demand for global tilapia production (and hence animal protein). Furthermore, there were even reports that tilapia have been successfully raised in seawater with survival of 84·1–93·5 %[9]. However, relatively few studies have been devoted to the differences in nutritional adaptation and nutritional requirement of GIFT in different salinity environments.

Comprehensive and balanced nutrition is indispensable for the optimum growth performance and maintenance of fish[10]. Lysine is an essential amino acid in fish due to its lack of

Abbreviations: DLL, dietary lysine level; FAS, fatty acid synthase; FCR, feed conversion ratio; GIFT, genetically improved farmed tilapia; GK, glucokinase; SGR, specific growth rate; SREBP1, sterol regulatory element-binding protein 1; TC, total cholesterol; TOR, target of rapamycin; S6K1, S6 kinase 1; 4E-BP1, 4E binding protein 1.

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self-synthesis capacity[11]. The shortage of lysine was accompanied by poor growth performance and feed utilisation in several fish species[12,13]. Indeed, lysine supplementation could reduce the body fat of fish[14] and lower dietary protein levels[15]. Furthermore, the activation of the target of rapamycin (TOR) signalling pathway could be influenced by lysine[16] and may induce growth hormone and insulin-like growth factor 1[17] which is possibly related to the growth-promoting effects of lysine.

Salinity is an important external factor for fish growth, as fish need to maintain osmotic homoeostasis when encountering different salinity ranges[18]. Previous researchers have evaluated that growth performance[19,20], body composition[21] and blood parameters[22] were closely associated with salinity. In a previous report, Rafael de Souza Romaneli *et al.*[23] revealed that the appropriate dietary lysine requirement for GIFT juveniles was estimated at 1·93–1·95 % of the dry diet. However, changes in salinity might change the energy utilisation of fish, thereby affecting nutritional requirements, probably due to the reorientation of physiological processes under altered salinity conditions[24]. For example, Wu *et al.*[25] and Xu *et al.*[26] elucidated that salinity could change the optimal protein and methionine requirements of GIFT, respectively. However, only a few studies have been devoted to stimulating nutrient metabolism by salinity[27,28]. While lysine requirements and nutritional metabolism of GIFT under different salinities are still unclear. Therefore, this research extended the knowledge of the dietary lysine requirements at two cultured salinities and explored the effects of dietary lysine level (DLL) and salinity on the growth performance and nutrient metabolism of GIFT juveniles which might be established as a theoretical basis for the development of a particular feed for different salinity environments.

Materials and methods

The experimental protocol was followed by the Institutional Animal Care and Ethics Committee of Nanjing Agricultural University, Nanjing, China (Permit number: SYXK (Su) 20110036).

Experimental diet preparation

Six isonitrogenous and isoenergy purified diets with 16·4 kJ/g metabolisable energy and 33·0 % crude protein were formulated, containing graded lysine levels (1·34, 1·70, 2·03, 2·41, 2·72 and 3·04 % of dry diets) at two salinity levels (0 ‰, freshwater; 8 ‰, brackish water). The primary protein sources were soyabean meal and fish meal, while the primary sources of fat were soyabean oil and soya lecithin (online Supplementary Table S1). The whole-body amino acid profile of GIFT was simulated by supplementation of diets with crystalline L-amino acids (online Supplementary Table S2). Feed raw ingredients were shattered with a small feed grinder and then adequately blended with soyabean oil and water. The granulation process was performed by a pelletiser (F-26 (II), South China University of Technology, China) and then air-dried to approximately 10 % moisture. The pellet feed was sealed into bags and stored at −18°C before the trial initiation.

Experimental procedure

Juvenile GIFT was obtained from the Yixing Experiment Base of the Freshwater Fishery Research Center (Wuxi, China), and all fish were acclimated for 2 weeks under experimental conditions. Before starting the formal feeding experiments, fish were fasted for 24 h and weighed. Then, 720 lively and even size (initial weight 6·24 (SEM 0·01) g) fish were randomly allocated into thirty-six recirculating water aquaculture tanks (180 l) with twenty fish per tank. The experimental fish were evenly divided into twelve groups with triplicate tanks, and 0 ‰ salinity and 8 ‰ salinity were adjusted to six groups as described in our previous study[26]. Fish were fed to apparent visual satiation three times daily (07.00, 12.00 and 17.00 hours). During the 62-d trial, the water temperature (25 (SEM 1)°C) and dissolved oxygen (6–7 mg/l) were monitored daily and maintained. Cultured salinity during the experiment was maintained by seawater crystals and measured daily using a salinity meter (ATAGO S-10E).

Sampling and chemical analysis

Sample collection. After an 8-week feeding trial, all fish were starved 24 h from the last feeding day, and then each barrel of fish was bulk-weighed and counted. Then, five fish were randomly selected, and two fish were stored in a −20°C freezer for the analysis of the whole-body composition. Simultaneously, samples of the liver were also collected from the other fish. Blood samples were obtained from the tail vein of five fish, and plasma was obtained by centrifugation (4000 × ***g***, 15 min, 4°C). Finally, all samples of plasma and tissues were frozen at −80°C.

Biochemical analysis. Proximate analysis of raw materials, diets and whole-body composition were conducted using the established methods of AOAC[29]. The principal detection equipment and methods are summarised in online Supplementary Table S3. The amino acid composition of ingredients was referred to our previous study[30].

Differential expression analyses of the genes were performed with real-time PCR analysis. First, the total RNA of the experimental fish was isolated from liver tissue using the RNAiso plus kit (Takara). Then, the quantity and purity of RNA were checked using spectrometric methods, and real-time PCR was performed with a 7500 real-time PCR machine using the One-Step SYBR® PrimeScript® Plus RT-PCR Kit. The specific primers for the target genes are shown in online Supplementary Table S4. *β*-actin was employed as a non-regulated reference gene, and no changes of *β*-actin gene expression were observed in our research. Finally, the expressions of target genes were calculated based on a mathematical model by Pfaffl[31].

Protein extract was isolated using RIPA lysis buffer (50 mM Tris, 150 mM NaCl, 1 % TritonX-100, 0·1 % SDS, 1 % sodium deoxycholate, 1 mM EDTA). Protease and phosphatase inhibitors were added to all buffers before experiments. Western blot analysis was conducted according to the procedures as previously described[26]. Briefly, protein concentrations were determined by the BCA method (BB-3401, BestBio). Tissue lysates were separated by SDS-PAGE gel and then washed with Tris Buffered Saline with Tween 20 (TBST) at room temperature. Primary antibodies were incubated overnight at 4°C and then

incubated with the appropriate secondary antibody for 1 h. mTOR (#2972), phosphor-mTOR (#2971), 4E binding protein 1 (4E-BP1) (#9452) and phosphor-4E-BP1 (#9459) were obtained from Cell Signaling Technology Inc. β-actin (#ab0035) was obtained from Abways Technology. S6 kinase 1 (S6K1) (#1485–1-AP) was obtained from Proteintech Group. After incubation, TBST was used to wash the membrane. Specific proteins were visualised by Enhanced Chemiluminescence (ECL) luminescent solution. Band intensities were measured using a chemiluminescence imaging system (Clinx).

Statistical analysis

All data were presented as the means with their standard error of the means, and $P < 0{\cdot}05$ was regarded as statistically significant. The effect of DLL and salinity and their interaction were analysed by two-way ANOVA, significant interaction between the main effects was observed for variables and a one-way ANOVA was then used as an aid for the interpretation of data. Then, Tukey's multiple comparisons were assessed by SPSS 26.0 software (IBM SPSS). Meanwhile, after estimating the coefficients (R^2) of different mathematical models (broken-line regression model[32] and quadratic regression model[33]), the quadratic regression model was selected to estimate the optimum dietary lysine supplementation level in the practical diet.

Results

Growth performance

The growth and feed utilisation parameters of fish are presented in Table 1. Fish promptly accepted experimental diets, and the survival rate of GIFT was independent of DLL (1·34–3·04 %) and salinity. As shown, the specific growth rate (SGR) and weight gain rate were significantly affected by salinity and DLL, which increased significantly with the improvement of DLL up to 2·41 % and then decreased ($P < 0{\cdot}05$). Moreover, higher weight gain rate and SGR were observed in 8 ‰ salinity level than 0 ‰ salinity level; however, DLL did not influence growth performance at 8 ‰ salinity. The lowest feed conversion ratio (FCR) was observed in 2·41 % DLL group, and a lower FCR was observed in 8 ‰ salinity level than 0 ‰ salinity level. From the perspective of lysine level, 8 ‰ salinity compared with 0 ‰ salinity had no significant influence on FCR ($P \geq 0{\cdot}05$). According to the quadratic regression model of SGR and FCR, the recommended range of lysine requirement of GIFT under different salinity was 2·03–2·20 % (0 ‰) and 2·20–2·41 % (8 ‰) (Fig. 1 and 2).

Whole-body composition

The whole-body composition results are presented in Table 2. The whole-body moisture, crude protein, lipid and ash content of GIFT were significantly affected by salinity ($P < 0{\cdot}05$), and crude protein and lipid were also affected by DLL. In addition, all composition indicators were significantly higher at 0 ‰ salinity than 8 ‰ salinity except for moisture ($P < 0{\cdot}05$). The whole-body lipid content was higher in the treatment group fed 1·34 % DLL than other groups. Compared with lipid, the protein content was higher in the treatment groups with 2·03 and 2·41 % DLL, and the maximal protein content appeared at 2·03 % (0 ‰) and 2·41 % (8 ‰). Moreover, there was no significant difference among ash by DLL, and no interaction effects between DLL and salinity were detected in the whole-body composition.

Plasma biochemical analysis

The results of glucose (GLU), triacylglycerol (TAG), total protein (TP) and total cholesterol (TC) in plasma are presented in Table 3. TAG concentration was not affected by salinity ($P \geq 0{\cdot}05$) but was higher in fish fed 2·41 % DLL than in the other groups. Meanwhile, there was no significant difference in the levels of TC and total protein between DLL ($P \geq 0{\cdot}05$), whereas salinity levels were positively related to TC and total protein levels. Glucose was not influenced significantly by DLL ($P \geq 0{\cdot}05$) but was inversely associated with increasing salinity ($P < 0{\cdot}05$). Furthermore, no interaction was found between DLL and salinity ($P \geq 0{\cdot}05$).

Relative gene expressions

The results of TOR pathway-related genes in the liver of GIFT fed with different levels of lysine at two salinities are presented in Fig. 3. The expressions of TOR (Fig. 3(a)), ribosomal protein S6K1 (Fig. 3(b)) and eukaryotic initiation factor 4E-BP1 (Fig. 3(c)) were affected significantly by DLL and salinity. The TOR and S6K1 expression levels initially increased and then decreased with increasing DLL, reaching maximum mRNA level in both 2·41 % DLL and the maximum mRNA level of 4E-BP1 in 2·01 % DLL (0 ‰) and 2·41 % DLL (8 ‰). Additionally, a significantly higher expression of TOR was presented in the 8 ‰ salinity level than 0 ‰ salinity level ($P < 0{\cdot}05$). Meanwhile, the expressions of S6K1 and 4E-BP1 had a similar trend with TOR. Furthermore, there were significant interactions among TOR, S6K1 and 4E-BP1 expression levels between DLL and salinity ($P < 0{\cdot}05$). 8 ‰ salinity improved the protein and its phosphorylation protein levels of mTOR when compared with 0 ‰ salinity groups. The protein levels of SK61 and 4E-BP1 had the similar trends (Fig. 3(d)). Moreover, higher DLL groups (2·41 ‰, 2·72 ‰ and 3·04 ‰) have higher protein and its phosphorylation protein levels of mTOR in 8 ‰ salinity level.

The relative expressions of glucose metabolism-related genes in the liver of GIFT are presented in Fig. 4. Glucokinase (GK) (Fig. 4(a)) and glucose-6-phosphatase (G6 Pase) (Fig. 4(b)) expression levels were significantly influenced by DLL, salinity and their interaction ($P < 0{\cdot}05$). Moreover, the expressions of GK and G6 Pase were significantly higher at 0 ‰ salinity than 8 ‰ salinity ($P < 0{\cdot}05$). Meanwhile, the expressions of GK and G6 Pase first increased and then reached a maximum at 2·41 % DLL. The expression level of glucose transporter 2 (GLUT2) (Fig. 4(c)) was not influenced by DLL at 0 ‰ salinity ($P \geq 0{\cdot}05$), while the GLUT2 expression level was significantly higher in the 2·41 % DLL group than the other groups at 8 ‰ salinity. Additionally, the expression level of GLUT2 was decreased with increasing salinity ($P < 0{\cdot}05$), and there was no significant interaction between DLL and salinity ($P < 0{\cdot}05$).

Hepatic fatty acid synthase (FAS) (Fig. 5(a)) and sterol regulatory element-binding protein 1 (SREBP1) (Fig. 5(b)) expression levels were significantly affected by salinity and DLL. There were

Table 1. Growth performance and feed utilisation of GIFT juvenile fed with diets containing six levels of dietary lysine at two salinities for 62 d (Mean values with their standard errors of the mean)

	Parameters											
Experimental groups	AIW (g)		AFW (g)		WGR (%)		FCR		SGR (%/d)		SR (%)	
Salinity (‰) /Lysine levels (%)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0/1·34	6·24	0·01	55·86	1·17^{a}	794·70	20·32^{ab}	1·13	0·04^{bc}	3·53	0·06^{ab}	95·0	2·89
0/1·70	6·26	0·02	60·02	1·00^{bc}	858·23	16·11^{abc}	1·10	0·03^{abc}	3·64	0·05^{abc}	91·7	4·41
0/2·03	6·26	0·02	62·16	0·24^{cd}	892·70	6·50^{bc}	1·03	0·04^{a}	3·70	0·02^{bc}	91·7	6·01
0/2·41	6·25	0·01	62·77	1·98^{d}	904·20	34·05^{c}	1·06	0·02^{ab}	3·72	0·09^{c}	93·3	4·41
0/2·72	6·25	0·02	56·59	0·53^{ab}	805·20	12·50^{abc}	1·10	0·04^{bc}	3·55	0·03^{abc}	98·3	1·67
0/3·04	6·25	0·01	54·92	0·52^{bc}	779·20	11·40^{a}	1·14	0·05^{c}	3·51	0·03^{a}	95·0	2·89
8/1·34	6·24	0·01	59·78	0·54	858·33	8·65	1·08	0·02	3·65	0·03	100·0	0·00
8/1·70	6·25	0·01	60·86	0·65	874·30	10·85	1·08	0·03	3·67	0·03	96·7	3·33
8/2·03	6·24	0·01	62·40	1·98	899·80	31·76	1·06	0·03	3·71	0·09	96·7	1·67
8/2·41	6·24	0·01	63·68	2·45	920·70	39·07	1·06	0·05	3·75	0·11	91·7	4·41
8/2·72	6·25	0·01	62·77	1·88	905·20	18·74	1·05	0·02	3·72	0·05	96·7	1·67
8/3·04	6·25	0·01	60·63	0·76	869·47	10·44	1·07	0·03	3·66	0·03	100·0	0·00
Salinity (‰)												
0	6·25	0·02	59·09	0·89^{x}	844·89	14·30^{x}	1·09	0·01^{y}	3·64	0·09^{x}	94·17	6·24
8	6·24	0·02	61·76	2·57^{y}	889·71	9·95^{y}	1·06	0·01^{x}	3·70	0·07^{y}	96·94	4·58
Lysine level (%)												
1·34	6·25	0·02	57·30	1·12^{a}	826·52	17·32^{a}	1·11	0·02^{b}	3·59	0·03^{a}	97·50	4·18
1·70	6·26	0·03	60·44	0·57^{bc}	866·27	9·40^{bc}	1·09	0·01^{bc}	3·66	0·02^{bc}	94·17	6·65
2·03	6·26	0·03	62·28	0·90^{bc}	896·25	14·58^{bc}	1·04	0·01^{a}	3·71	0·02^{bc}	94·17	7·36
2·41	6·25	0·02	63·22	1·43^{b}	923·26	25·51^{b}	1·06	0·02^{bc}	3·73	0·04^{b}	92·50	6·89
2·72	6·25	0·02	60·30	1·63^{bc}	865·20	26·85^{bc}	1·07	0·02^{bc}	3·65	0·05^{bc}	97·50	2·74
3·04	6·25	0·02	58·34	1·47^{bc}	833·36	23·12^{a}	1·10	0·02^{b}	3·60	0·04^{a}	97·50	4·18
Two-way ANOVA												
Salinity	ns		**		**		*		**		ns	
Lysine	ns		**		**		*		**		ns	
Interaction	ns		ns		ns		ns		ns		ns	

GIFT, genetically improved farmed tilapia; WGR, weight gain rate; FCR, feed conversion ratio; SGR, specific growth rate; AIW, average initial weight; AFW, average final weight; SR, survival rate.
Values are mean of three replicates ± standard error of the mean. Means in the same column with different superscripts^{abcd} are significantly different ($P < 0·05$), superscripts^{xy} denote a significant $P < 0·05$ difference in values between 0 ‰ and 8 ‰ salinity as determined by *t* test.
* Means $P < 0·05$.
** Means $P < 0·01$, 'ns' means $P \geq 0·05$. Means with the same letters or absence of letters indicate not significantly different between treatments.
WGR (%) = 100 × (final body weight (g) - initial body weight (g))/initial body weight (g).
FCR = dry feed fed (g)/(final body weight (g) - initial body/weight (g)).
SGR (% day^{-1}) = 100 × ((ln (final body weight (g)) – ln (initial body weight (g)))/days).
SR (%) = 100 × (Number of fish survived/Total number of fish stocked).

significantly high expressions of FAS and SREBP1 at 8 % salinity ($P < 0·05$). Moreover, the expression of FAS and SREBP1 was decreased with increasing DLL at 0 ‰ salinity, and then the minimum values were shown in 2·72 % and 3·04 % DLL groups, respectively. Similarly, the gene expression of FAS and SREBP1 showed a general downward trend in the 8 ‰ salinity groups except for 2·41 % DLL group. Moreover, the expression levels of FAS and SREBP1 exhibited a significant interaction between DLL and salinity ($P < 0·05$).

Discussion

Lysine is commonly considered the first limiting essential amino acid in plant protein sources utilised in many fish foodstuffs, and lysine was the most abundant essential amino acid found in many fish species[34,35]. The present results indicated that DLL and salinity had a remarkable effect on the growth performance and feed utilisation of GIFT, which were shown by SGR and FCR. Moreover, there was a marked decline in growth performance when DLL was greater than 2·41 % at 0 ‰ and 8 ‰ salinity. Therefore, this result showed that lysine played a crucial role in the normal growth of GIFT juveniles. Similarly, previous studies have demonstrated that the optimum growth performance of some fish, including Nile tilapia, grass carp (*Ctenopharyngodon idellus*) and turbot (*Scophthalmus maximus*), could be significantly affected by DLL[23,36,37]. Salinity was also an essential factor in fish growth because an improper salinity environment could change growth, macronutrient selection in fish and even cause death[19,22,38]. In the present study, fish living in 8 ‰ salinity environment showed a relatively good growth performance. This may elucidate that GIFT might have been more inclined to accept brackish water. Compared with the hypertonic environment, fish in isotonic environments could store more energy to use for growth, and Qiang *et al.*[8] have found that 8 ‰ salinity near the isotonic state of GIFT. Similarly, our results parallel those reported by Likongwe *et al.*[39] and González-Félix *et al.*[40], in which the highest growth rate of fish was observed at salinities close to the isosmotic point compared with freshwater and seawater, which might also be the explanation why GIFT juveniles have higher growth performance under 8 ‰ salinity.

Additionally, according to SGR and FCR, the quadratic regression analysis illustrated that the optimum requirement DLL of

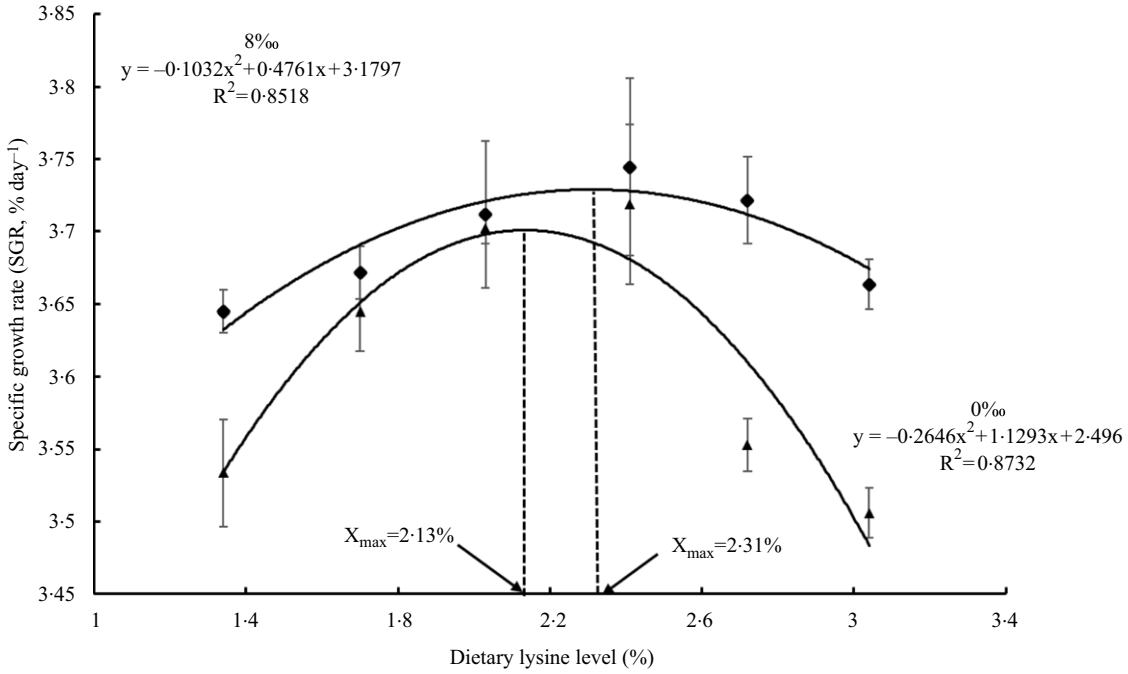

Fig. 1. Quadratic regression model analysis of specific growth rate (SGR) against graded different levels of dietary lysine at (▲) 0 ‰ and (◆) 8 ‰ salinity.

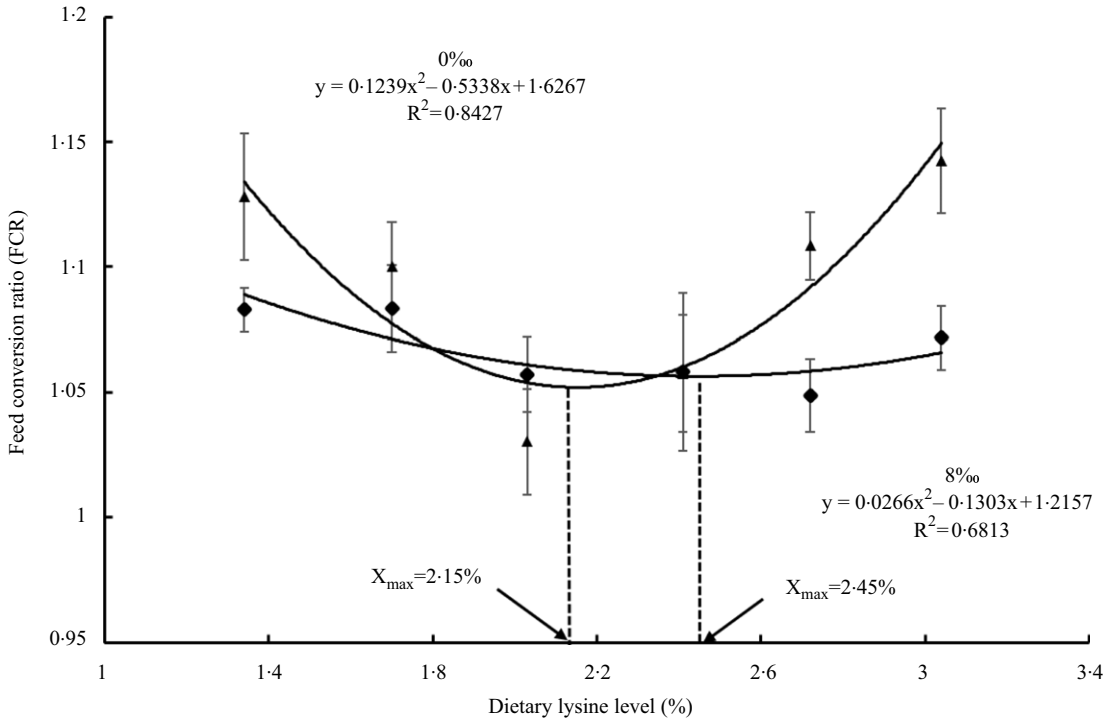

Fig. 2. Quadratic regression model analysis of feed conversion ratio (FCR) against graded different levels of dietary lysine at (▲) 0 ‰ and (◆) 8 ‰ salinity.

GIFT cultured in 8 ‰ salinity was higher than in freshwater, which was 2·31 %, 2·45 % (8 ‰), and 2·13 %, 2·15 % (0 ‰), respectively, possibly related to the requirement for more protein in fish at high salinity. For example, Larumbe-Morán *et al.*[41] and Wu *et al.*[25] both found that GIFT juveniles might need more protein to satisfy the demand of growth in brackish water. Moreover, 8 ‰ salinity also significantly elevated the plasma content of total protein in this study. Generally, it was considered that the essential amino acid needs for protein accretion correspond to the amino acid content of tissue protein gain[42]. Similarly, the present study indicated that a higher DLL of GIFT might be required to maintain growth needs at

Table 2. Whole-body composition of GIFT juvenile fed with diets containing six levels of dietary lysine at two salinities for 62 d (Mean values with their standard errors of the mean)

Experimental groups	Parameters							
Salinity (‰) /lysine levels (%)	Moisture (%)		Protein (%)		Lipid (%)		Ash (%)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0/1·34	70·68	0·32	14·24	$0·23^{a}$	8·12	$0·11^{b}$	3·93	$0·09^{ab}$
0/1·70	72·29	0·38	14·23	$0·18^{a}$	7·22	$0·19^{ab}$	3·89	$0·06^{ab}$
0/2·03	70·87	0·57	15·88	$0·31^{b}$	6·63	$0·42^{a}$	3·67	$0·13^{a}$
0/2·41	70·86	1·34	15·34	$0·18^{b}$	6·98	$0·54^{ab}$	3·91	$0·14^{ab}$
0/2·72	71·60	0·47	15·53	$0·26^{b}$	6·67	$0·36^{a}$	3·86	$0·07^{ab}$
0/3·04	71·02	0·70	15·65	$0·22^{b}$	6·65	$0·57^{a}$	4·03	$0·06^{b}$
8/1·34	67·85	1·83	15·78	0·66	9·46	$0·49^{b}$	4·24	0·12
8/1·70	69·85	0·74	15·52	0·31	8·30	$0·12^{ab}$	4·36	0·44
8/2·03	69·14	1·48	16·70	0·87	8·43	$0·57^{ab}$	4·29	0·26
8/2·41	67·99	0·26	17·19	0·08	8·71	$0·39^{ab}$	4·17	0·06
8/2·72	68·70	0·96	16·50	0·93	8·68	$0·17^{ab}$	4·15	0·01
8/3·04	69·69	0·96	16·51	0·56	7·62	$0·74^{a}$	4·14	0·08
Salinity (‰)								
0	71·25	$0·29^{y}$	15·27	$0·23^{x}$	7·00	$0·18^{x}$	3·88	$0·04^{x}$
8	68·95	$0·45^{x}$	16·43	$0·30^{y}$	8·60	$0·20^{y}$	4·22	$0·07^{y}$
Lysine level (%)								
1·34	69·26	1·11	15·01	$0·47^{ab}$	8·92	$0·42^{b}$	4·08	0·11
1·70	71·31	0·68	14·88	$0·33^{a}$	7·65	$0·29^{ab}$	4·08	0·18
2·03	70·00	0·81	16·29	$0·45^{b}$	7·53	$0·51^{ab}$	3·98	0·19
2·41	69·71	1·02	16·27	$0·42^{b}$	7·85	$0·49^{ab}$	4·01	0·10
2·72	70·44	0·81	16·01	$0·48^{ab}$	7·48	$0·53^{ab}$	3·98	0·08
3·04	70·36	0·61	16·08	$0·33^{ab}$	7·13	$0·47^{a}$	4·09	0·05
Two way ANOVA								
Salinity	**		**		**		**	
Lysine	ns		*		*		ns	
Interaction	ns		ns		ns		ns	

GIFT, genetically improved farmed tilapia.
Values are mean of three replicates ± standard error of the means. Means in the same column with different superscripts^{abcd} are significantly different ($P < 0·05$), superscripts^{xy} denote a significant $P < 0·05$ difference in values between 0 and 8 ‰ salinity as determined by *t* test.
* Means $P < 0·05$.
** Means $P < 0·01$, 'ns' means $P \geq 0·05$. Means with the same letters or absence of letters indicate not significantly different between treatments.

8 ‰ salinity. However, no convincing evidence shows that protein requirements are affected by environmental factors (e.g. salinity)[43,44], which still needs further investigation and confirmation.

In previous research, Prabu *et al.*[45] reported that DLL did not influence the whole-body composition of juvenile GIFT. Nevertheless, in the present study, DLL significantly affected whole-body crude protein content, and in fish fed 2·03 %, which showed the maximum value at 0 ‰ salinity groups. Generally, protein deposition seemed to govern live weight gain in fish[46]. This result indicated that proper lysine addition might promote protein deposition. Similar findings were also found in other fish, such as Nile tilapia[23] and large yellow croaker (*Pseudosciaena crocea*)[47]. These variations in whole-body composition might include size, age, dietary constituents, feeding strategy, rearing environment and research design[48]. Moreover, whole-body moisture and ash content were not affected by dietary lysine supplementation. Similar results were also previously observed in large yellow croaker and grass carp by Zhang *et al.*[47] and Li *et al.*[49]. In addition, a higher crude protein content of GIFT juveniles was observed at 8 ‰ salinity than 0 ‰ salinity, which was in agreement with the study of Wu *et al.*[25] on GIFT. Increasing crude protein content in the brackish water might result from lower energy requirements for osmoregulation than in 0 ‰ salinity, indicating that the fish have more energy to use for protein deposition under 8 ‰ salinity. Of the major energy-yield nutrients, lipids have the greatest energy density. In the present study, an overall downward trend was detected in whole-body lipid content with increasing DLL, which corresponded well to the study of Mai *et al.*[13], who found that the whole-body lipid content significantly decreased with increasing DLL in juvenile Japanese seabass. Furthermore, Deng *et al.*[50] found that juvenile Pacific threadfin (*Polydactylus sexfilis*) showed higher lipid deposition in groups fed the lower DLL, and the authors hypothesised that deficiency in DLL impaired lipogenesis metabolism. Meanwhile, the whole-body lipid content in the present study was positively affected by 8 ‰ salinity. Correspondingly, Liu *et al.*[51] reported that American shad (*Alosa sapidissima*) at 7 ‰ salinity had a higher lipid content than freshwater. In summary, the lower DLL at the higher salinity might induce lipid deposition in the fish body.

TOR signalling is a key nutrient-sensing pathway that plays a crucial role in inducing protein synthesis through the regulation of 4EBP1 and S6K1 phosphorylation[52]. Previous research has found that DLL could stimulate protein synthesis in fish by activating the TOR pathway such as largemouth bass (*Micropterus salmoides*)[14] and juvenile turbot[53]. Similarly, significantly enhanced effects were observed in the expression of TOR pathway-related genes by DLL and showed a similar trend of whole-body protein content in juvenile GIFT, indicating that the

Table 3. Plasma biochemical composition of GIFT tilapia juvenile fed with diets containing six levels of dietary lysine at two salinities for 62 d (Mean values with their standard errors of the mean)

Experimental groups	Parameters							
Salinity (‰)/lysine levels (%)	GLU (mmol/l)		TP (g/l)		TAG (mmol/l)		TC (mmol/l)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0/1·34	12·65	0·72	23·32	0·43	58·43	5·76^{ab}	2·69	0·12
0/1·70	12·99	1·07	24·07	0·87	51·76	1·17^{a}	2·92	0·15
0/2·03	13·91	1·03	23·84	0·92	55·16	3·62^{ab}	2·61	0·07
0/2·41	13·43	1·15	22·88	0·65	63·85	2·45^{b}	2·84	0·09
0/2·72	9·99	0·66	24·33	0·92	49·18	2·60^{a}	2·93	0·19
0/3·04	10·72	0·56	23·04	0·74	51·22	5·20^{a}	3·04	0·35
8/1·34	8·85	0·39	27·72	1·18	54·40	2·78^{a}	3·19	0·23
8/1·70	8·87	0·90	25·48	1·18	64·47	3·09^{b}	3·23	0·18
8/2·03	9·68	1·78	26·15	0·59	52·15	2·49^{a}	3·50	0·07
8/2·41	9·22	2·08	28·19	0·74	66·94	2·97^{b}	3·86	0·23
8/2·72	6·38	0·31	26·34	0·91	48·30	2·89^{a}	3·33	0·13
8/3·04	6·82	0·06	27·74	0·69	46·34	3·86^{a}	3·25	0·19
Salinity (‰)								
0	12·20	0·43^{y}	23·58	0·30^{x}	55·21	1·68	2·84	0·07^{x}
8	8·55	0·57^{x}	26·94	0·39^{y}	54·50	1·90	3·39	0·08^{y}
Lysine level (%)								
1·34	10·54	0·76	25·52	0·94	55·91	2·62^{ab}	2·94	0·15
1·70	11·16	0·99	24·77	0·73	58·12	3·20^{bc}	3·07	0·12
2·03	11·56	1·27	25·00	0·64	53·82	2·23^{ab}	3·06	0·16
2·41	10·80	1·51	25·54	1·00	65·01	1·84^{c}	3·35	0·20
2·72	8·44	0·82	25·34	0·70	48·79	1·81^{a}	3·13	0·13
3·04	9·25	0·79	25·39	0·92	48·43	3·01^{a}	3·15	0·19
Two-way ANOVA								
Salinity	**		**		ns		**	
Lysine	ns		ns		**		ns	
Interaction	ns		ns		ns		ns	

GIFT, genetically improved farmed tilapia; GLU, glucose; TP, total protein; TAG, triacylglycerol; TC, total cholesterol.
Values are mean of three replicates ± standard error of the means. Means in the same column with different superscripts^{abcd} are significantly different ($P < 0·05$), superscripts^{xy} denote a significant $P < 0·05$ difference in values between 0 and 8 ‰ salinity as determined by *t* test.
** Means $P < 0·01$, 'ns' means $P \geq 0·05$. Means with the same letters or absence of letters indicate not significantly different between treatments.

balance of DLL could improve the protein synthesis of GIFT. Additionally, compared with 0 ‰ salinity, the expression of hepatic TOR pathway-related gene levels was higher in 8 ‰ salinity at the same lysine level, indicating that the protein synthesis metabolism of GIFT was more vigorous under 8 ‰ salinity. Wu *et al.*[25] reported similar results in GIFT juveniles. The catabolism of protein provides an important energy source for fish to adapt to environments[54]. Hence, this phenomenon might be due to the priority of protein used as the main energy source of GIFT juveniles. Furthermore, 8 ‰ salinity improved the protein and its phosphorylation protein levels of mTOR, and the protein levels of SK61 and 4E-BP1 had the similar trends. This was consistent with measurements of gene expression. Xu *et al.*[26] found similar results in a study regarding salinity and methionine level. Interestingly, higher DLL (2·41 ‰, 2·72 ‰ and 3·04 ‰) had a relatively higher protein and phosphorylation levels in 8 ‰ salinity. The above aspects might also explain why the lysine requirement was higher in brackish water than in freshwater environments.

Glycolysis is the route of glucose catabolism in animals, including fish, and GK is a key rate-limiting enzyme in glucose utilisation[55,56]. In the present experiments, GIFT juveniles fed 2·41 % DLL showed the highest hepatic GK mRNA levels and increased significantly with increasing salinity, indicating that appropriate DLL could increase glycolysis in the liver. Additionally, the positive result was observed in grass carp by Huang *et al.*[57]. GLUT2 specifically diffuses glucose and plays an essential role in the bidirectional transport of glucose to cells[58,59]. In this study, the expression of GLUT2 was positively influenced by salinity but was not affected by DLL. Song *et al.*[7] reported that a significantly higher expression of GLUT2 in Nile tilapia in brackish water (10 ‰) groups compared with freshwater. Likewise, GLUT2 gene expression paralleled changes in plasma glucose levels, which probably resulted from shifting a priority for glucose utilisation. Furthermore, gluconeogenesis is another vital pathway of glycometabolism in maintaining glucose homoeostasis, and the last and rate-limiting step of gluconeogenesis is regulated by G6 Pase[60,61]. The current study showed that 8 ‰ salinity significantly lowered the relative mRNA expression of G6 Pase. Similarly, G6 Pase enzyme activity changes were reported by Woo and Kelly[62] in sea bream (*Sparus sarba*). Interestingly, we found that both gluconeogenesis and glycolytic processes were simultaneously higher at 0 ‰ salinity. The reason might be that nutrient metabolism has been reorganised to maintain glucose homoeostasis and proper energy supplementation[63]. Correspondingly, we noticed that glucose levels were significantly lower at 8 ‰ salinity among the salinity treatments. Likewise, plasma glucose levels of juvenile turbot have been strongly affected by salinity[64]. Overall, these results implied that 0 ‰ salinity could boost carbohydrate

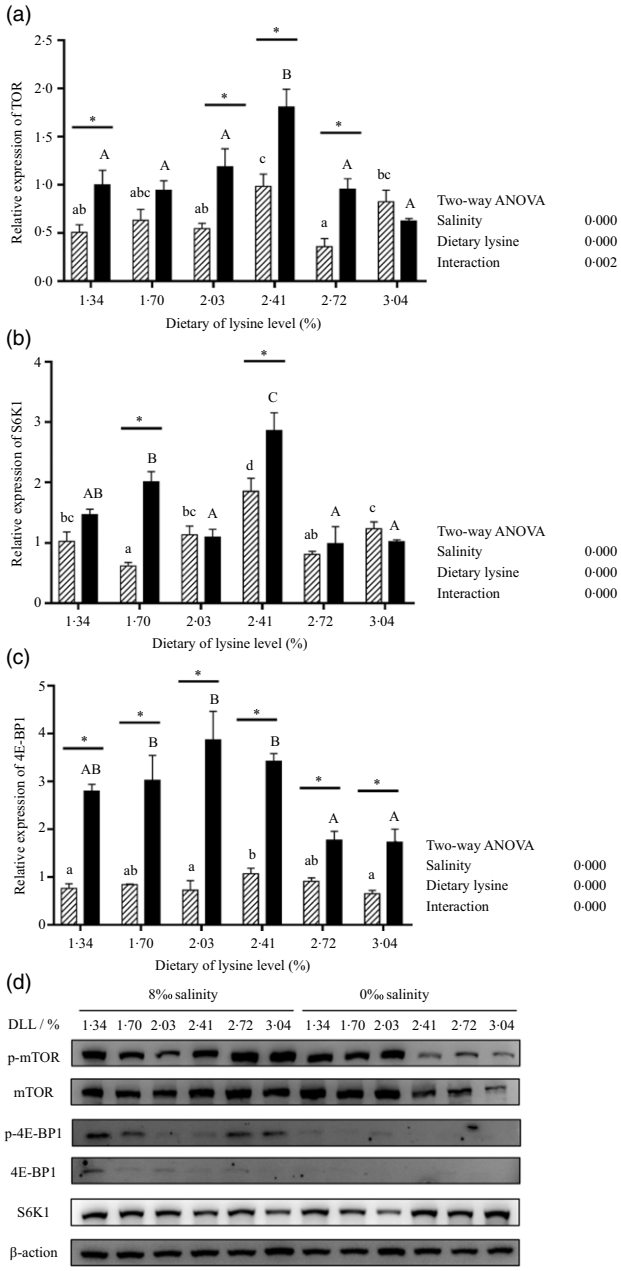

Fig. 3. Relative gene expressions of TOR signalling pathway in the liver of GIFT juvenile fed with six dietary lysine levels at (▨) 0 ‰ and (■) 8 ‰ salinity. (a) TOR, (b) S6K1, (c) 4E-BP1 and (d) Western blot analysis. Data are expressed as means with their standard error of the means, values with different superscripts are significantly different and 0‰ salinity labelled with lower case, 8‰ salinity labelled with upper case ($P < 0.05$). Means with the same letters or absence of letters indicate not significantly different between treatments. Asterisk (*) indicates a significant difference between 0 ‰ groups and 8 ‰ groups with $P < 0·05$. TOR, target of rapamycin; GIFT, genetically improved farmed tilapia; S6K, S6 kinase 1; 4E-BP1, 4E binding protein 1.

use and increase carbohydrate utilisation. Few examples have clarified the effect of salinity on glucose metabolism-related genes, and further mechanistic research is needed to elucidate these.

The whole-body lipid contents of GIFT showed that DLL and salinity could alter lipid deposition in fish. SREBP1 is responsible for the biosynthesis of fatty acids by regulating the FAS gene[65], which is a key enzyme and factor-catalysed lipogenesis pathway[66]. The current results suggested that the expressions of SREBP1 and FAS showed a similar tendency with increasing DLL, indicating that low DLL could increase the rate of lipid anabolism and that crystalline lysine supplementation might contribute to lower lipid deposition in GIFT juveniles. Similar results were also observed in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) by Espe *et al.*[67] and Cheng *et al.*[68]. Furthermore, related gene expression was consistent with the changing trend of whole-body crude lipid content of GIFT juveniles, which also supported our results.

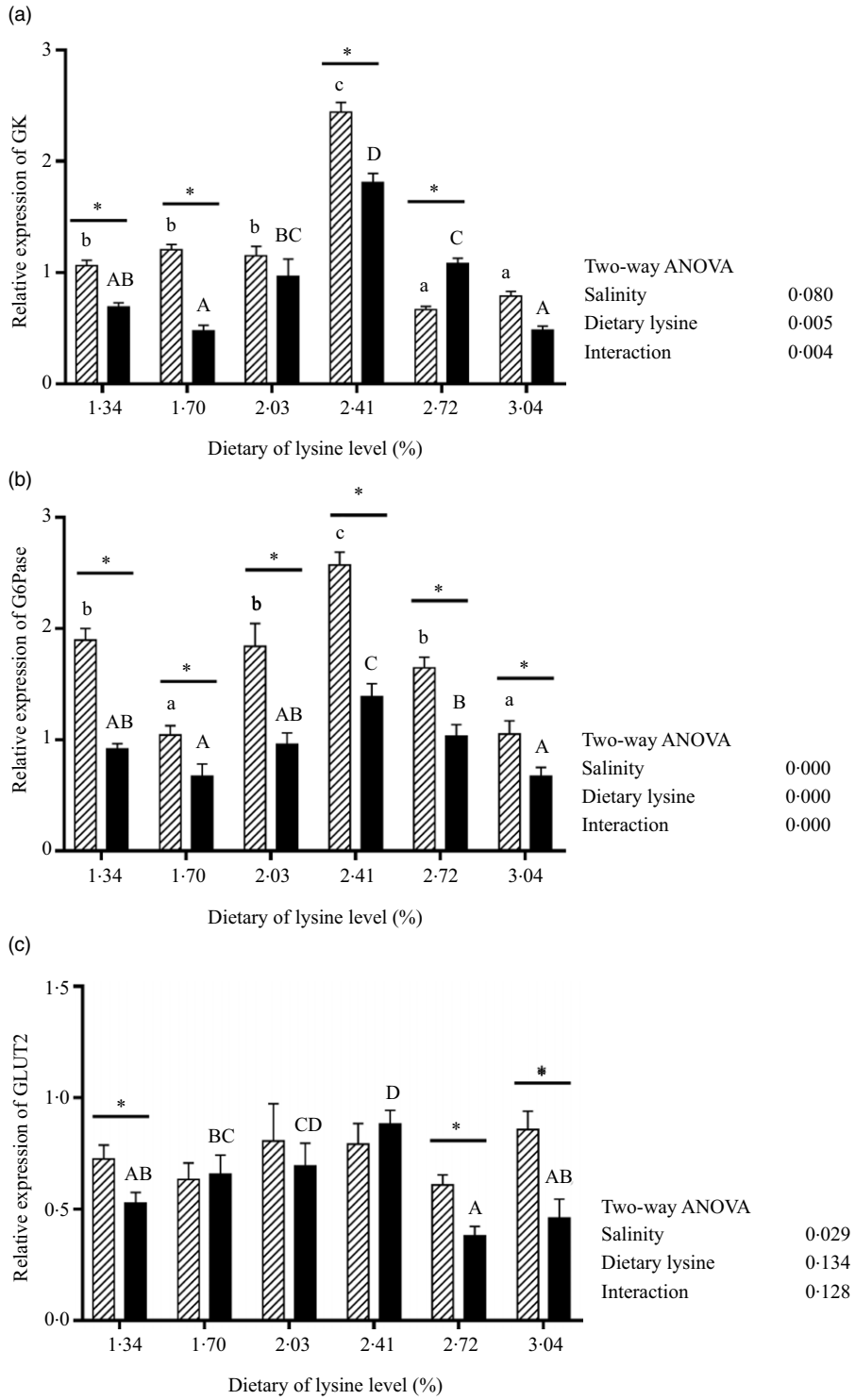

Fig. 4. Relative gene expressions of glycometabolism in the liver of GIFT juvenile fed with six dietary lysine levels at (▨) 0 ‰ and (■) 8 ‰ salinity. (a) GK, (b) G6 Pase and (c) GLUT2. Data are expressed as means with their standard error of the means, values with different superscripts are significantly different and 0‰ salinity labelled with lower case, 8‰ salinity labelled with upper case ($P < 0.05$). Means with the same letters or absence of letters indicate not significantly different between treatments. Asterisk (*) indicates a significant difference between 0 ‰ groups and 8 ‰ groups with $P < 0{\cdot}05$. GIFT, genetically improved farmed tilapia; GK, glucokinase; G6 Pase, glucose-6-phosphatase; GLUT2, glucose transporter 2.

Harpaz[69] has reported that carnitine could promote the oxidation of fat and reduce body fat in European sea bass (*Dicentrarchus labrax*).

As the precursor substance of carnitine synthesis, lysine levels affected carnitine levels in the body[70], which indicated that the increased lipid deposition in fish fed low-lysine diets might be

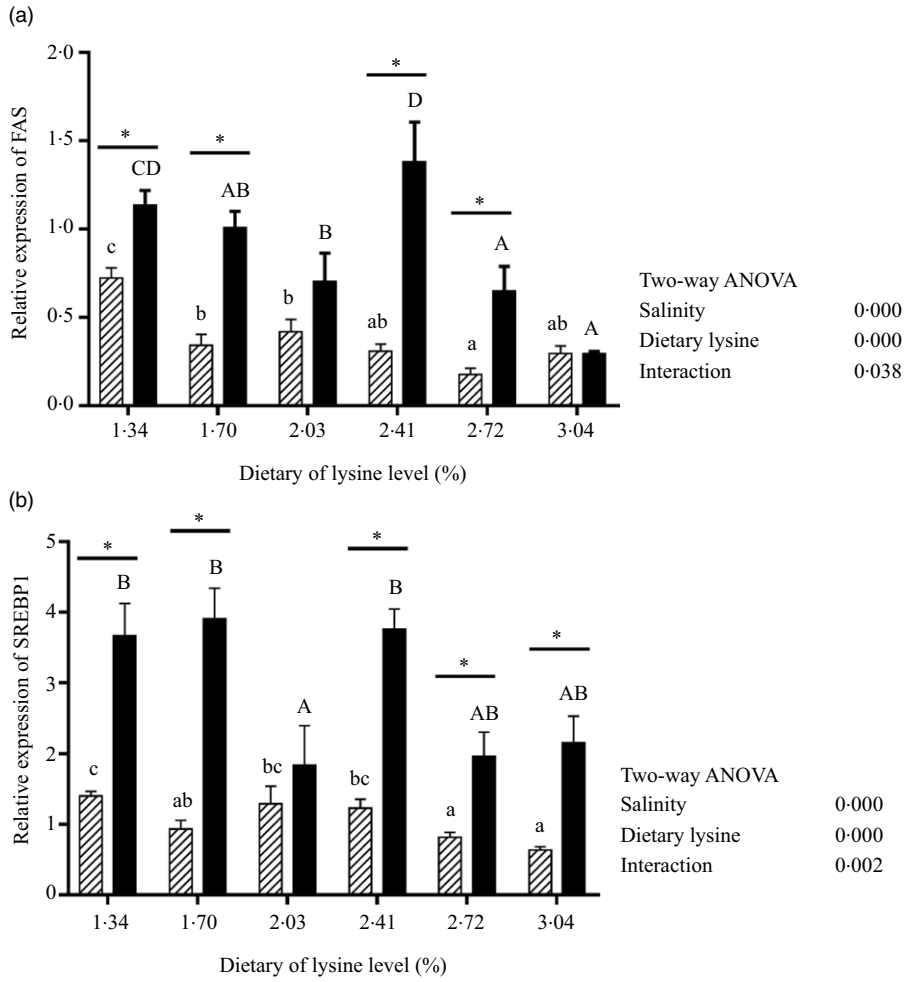

Fig. 5. Relative gene expressions of lipid synthesis in the liver of GIFT juvenile fed with six dietary lysine levels at (▨) 0 ‰ and (■) 8 ‰ salinity. (a) FAS and (b) SREBP1. Data are expressed as means with their standard error of the means, values with different superscripts are significantly different and 0‰ salinity labelled with lower case, 8‰ salinity labelled with upper case ($P < 0.05$). Means with the same letters or absence of letters indicate not significantly different between treatments. Asterisk (*) indicates a significant difference between 0 ‰ groups and 8 ‰ groups with $P < 0·05$. GIFT, genetically improved farmed tilapia; FAS, fatty acid synthase; SREBP1, sterol regulatory element-binding protein.

due to reducing carnitine[71]. Conversely, the up-regulated SREBP1 mRNA level was accompanied by up-regulated FAS mRNA levels when the salinity changed from 0 ‰ to 8 ‰. A similar phenomenon was also reported recently[26]. Therefore, this finding indicated that 8 ‰ salinity might enhance liver lipogenesis of GIFT. Additionally, the highest plasma TAG and TC values of GIFT were observed in the 8 ‰ salinity group. Similarly, Mylonas *et al.*[22] reported a reduction in TAG and TC levels when salinity changed from 4 ‰ salinity to 10 ‰ salinity in shi drum (*Umbrina cirrosa*). These results, coupled with a reduction in whole-body crude lipid content, further confirmed that 8 ‰ salinity could increase lipid deposition and lipogenic capacities.

Conclusions

In summary, according to the quadratic regression model of SGR and FCR, the recommended range of lysine requirement of GIFT under different salinity was 2·03–2·20 % (0 ‰) and 2·20–2·41 % (8 ‰). Furthermore, GIFT juveniles had higher growth performance at 8 ‰ salinity, and the TOR signalling pathway was further activated. Meanwhile, the gene expression levels of protein synthesis metabolism and lipid lipogenesis in the liver were more vigorous at 8 ‰ salinity. The GIFT juveniles contributed to enhanced carbohydrate utilisation at 0 ‰ salinity. However, lipids and proteins were deposited at 8 ‰ salinity.

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The authors declare no conflict of interest.

Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S0007114522001763

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