Effect of dietary arginine on growth, intestinal enzyme activities and gene expression in muscle, hepatopancreas and intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian)

Gangfu Chen[1], Lin Feng[1,2,3], Shengyao Kuang[1,4], Yang Liu[1,2,3], Jun Jiang[1,2,3], Kai Hu[1,2,3], Weidan Jiang[1,2,3], Shuhong Li[1], Ling Tang[1,4] and Xiaoqiu Zhou[1,2,3]*

[1]*Animal Nutrition Institute, Sichuan Agricultural University, Sichuan, Ya'an 625014, People's Republic of China*
[2]*Fish Nutrition and Safety in Production Sichuan University Key Laboratory, Sichuan Agricultural University, Sichuan, Ya'an 625014, People's Republic of China*
[3]*Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Sichuan Agricultural University, Sichuan, Ya'an 625014, People's Republic of China*
[4]*Animal Nutrition Institute, Sichuan Academy of Animal Science, Chengdu 610066, People's Republic of China*

Abstract

The present study was conducted to test the hypothesis that dietary arginine promotes digestion and absorption capacity, and, thus, enhances fish growth. This improvement might be related to the target of rapamycin (TOR) and eIF4E-binding protein (4E-BP). A total of 1200 juvenile Jian carp, *Cyprinus carpio* var. Jian, with an average initial weight of 6·33 (SE 0·03) g, were fed with diets containing graded concentrations of arginine, namely, 9·8 (control), 12·7, 16·1, 18·5, 21·9 and 24·5 g arginine/kg diet for 9 weeks. An real-time quantitative PCR analysis was performed to determine the relative expression of TOR and 4E-BP in fish muscle, hepatopancreas and intestine. Dietary arginine increased ($P < 0.05$): (1) glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities in muscle and hepatopancreas; (2) intestine and hepatopancreas protein content, folds height, and trypsin, chymotrypsin, lipase, Na$^+$/K$^+$-ATPase, alkaline phosphatase, γ-glutamyl transpeptidase and creatine kinase activities in intestine; (3) *Lactobacillus* counts; (4) relative expression of TOR in the muscle, hepatopancreas and distal intestine (DI); (5) relative expression of 4E-BP in proximal intestine (PI) and mid-intestine (MI), as compared with the control group. In contrast, dietary arginine reduced ($P < 0.05$): (1) plasma ammonia content; (2) *Aeromonas hydrophila* and *Escherichia coli* counts; (3) relative expression of TOR in PI and MI; (4) relative expression of 4E-BP in the muscle, hepatopancreas and DI. The arginine requirement estimated by specific growth rate using quadratic regression analysis was found to be 18·0 g/kg diet. These results indicate that arginine improved fish growth, digestive and absorptive ability and regulated the expression of *TOR* and *4E-BP* genes.

Key words: Arginine: Intestinal enzyme activities: Target of rapamycin: *Cyprinus carpio* var. Jian

Arginine is an essential amino acid for optimal fish growth[1]. Dietary arginine deficiency causes growth reduction and poor protein retention, as shown in coho salmon (*Oncorhynchus kisutch*), European sea bass (*Dicentrarchus labrax*) and Indian major carp (*Cirrhinus mrigala*)[2–4]. Protein deposition in fish is mainly associated with amino acid metabolism[5]. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are two important amino acid metabolic enzymes of fish[6]. Furthermore, ammonia was found to correlate with fish amino acid metabolism[7,8].

An increase in plasma ammonia nitrogen concentration was observed in the European sea bass fed with plant protein diets under a moderate or large excess of dietary arginine[9]. However, no study addressed the effects of arginine on GOT and GPT in fish. Recently, our laboratory reported that supplementation with methionine hydroxy analogue to practical diets decreased plasma ammonia levels and increased GOT and GPT activities in Jian carp (*Cyprinus carpio* var. Jian) hepatopancreas and muscle[10]. Accordingly, further studies are required to address the effect of arginine on amino acid metabolism in fish.

Fish growth rate is dependent on digestive and absorptive ability[11,12]. Digestion ability and absorption function were found to correlate with the growth and development of digestive organs[13]. Several studies[14–17] demonstrated that arginine and its intermediate had a beneficial influence on the pancreas and intestine by promoting tissue integrity and cell proliferation. However, studies on the effects of dietary arginine on the growth and development of fish digestive organs are limited. Digestion and absorption of nutrients depend on the activity of digestive enzymes and brush-border membrane enzymes[18]. Fish exocrine pancreas synthesises and secretes a large number of digestive enzymes into the intestinal lumen, such as trypsin, chymotrypsin, lipase and amylase[19,20]. Alkaline phosphatase (AKP), Na^+/K^+-ATPase and creatine kinase (CK) are considered to be involved in the absorption of nutrients in fish[21]. However, few studies have been conducted to investigate the effects of dietary arginine on fish intestinal enzyme activities. Synthesis and secretion of digestive enzymes from pancreatic exocrine tissue are sensitive to the redox state, which can be regulated by NO[22–25]. Moreover, studies have indicated that arginine residues have an important role in digestive and absorptive enzymes[26–32]. Studies from our laboratory have shown that glutamine, lysine and methionine improve digestive and brush-border membrane enzyme activities[33–35]. Hence, it is necessary to address the relationship between arginine and fish intestinal enzyme activities.

The intestinal microbiota contributes to host health status, and alterations in the microbial balance may produce detrimental effects in hosts[36,37]. A recent study has found that dietary methionine and protein improved *Lactobacillus* counts and reduced *Escherichia coli* and *Aeromonas* counts in juvenile Jian carp[35,38]. Furthermore, dietary arginine supplementation decreased the frequency of *Helicobacter* spp. and *Clostridium perfringens* in rabbit ileum[39]. However, few studies have evaluated the effects of arginine on fish intestinal microbial populations.

Protein synthesis is a key component of the processes involved in growth response[40]. The limiting step in protein synthesis is translation initiation, which is regulated by the signalling pathway of target of rapamycin (TOR) through eIF4E-binding protein (4E-BP) 1 and ribosomal protein S6 kinase[41]. *TOR* and *4E-BP* genes were cloned in our laboratory. Similarly, the mRNA expression of TOR decreased with dietary Thr and Trp levels in the intestine and muscle and increased with Gln supplementation in intestinal epithelial cells (IEC) of Jian carp (L Tang, L Feng and XQ Zhou, unpublished results). However, no study has addressed the effects of arginine on TOR and 4E-BP expression in fish tissues or organs. Moreover, the nutritional regulation of major kinases involved in the TOR pathway has been elucidated in fish. Re-feeding was found to enhance the phosphorylation of TOR in rainbow trout (*Oncorhynchus mykiss*) muscle and liver and promote the phosphorylation of 4E-BP1 in rainbow trout muscle[42,43]. Therefore, arginine might be related to the expression of *TOR* and *4E-BP* genes in fish, which needs to be investigated.

Jian carp is the first variety of common carp[44]. Its gross production is approximately more than 30 % greater than other varieties of common carp, and it has a high flesh quality[44,45]. Interestingly, it has become one of the most popular species for fish culture in China[46]. The present study was designed to test the hypothesis that dietary arginine promotes digestion and absorption capacity that can enhance Jian carp growth, which might be related to the expression of *TOR* and *4E-BP* genes.

Materials and methods

Experimental diets and procedure

The composition of the tested diets is given in Table 1. Fishmeal, rice gluten meal and crystalline amino acids were used as the main protein sources and were found to be limiting in arginine. Crystalline amino acids (Donboo Amino Acid, Nantong, Jiangsu, China) were used to simulate the amino acid profile of diets with 34 % whole chicken egg protein, except for arginine. The experimental diets were supplemented with L-arginine hydrochloride to provide arginine at the concentrations of 9·0, 12·0, 15·0, 18·0, 21·0 and 24·0 g/kg of diet. All diets were made iso-nitrogenous and iso-energetic (16·5 kJ/g of gross energy) with the addition of appropriate amounts of glycine. Zn, Fe, pyridoxine, pantothenic acid, inositol, riboflavin and thiamin were formulated to meet the nutrient requirements of Jian carp according to previous studies conducted in our laboratory[47–53]. The levels of other nutrients met the requirements for common carp according to the National Research Council[54]. The pH of each diet was adjusted to 7·0 by gradually adding 6·0 M-NaOH[10]. Pellets were produced and stored at − 20°C until use[55]. The arginine concentrations in experimental diets were determined to be 9·8 (control), 12·7, 16·1, 18·5, 21·9 and 24·5 g arginine/kg diet, as described by Wu *et al.*[56] using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA).

All experimental protocols were approved by the Animal Care Advisory Committee of Sichuan Agricultural University. Juvenile Jian carp were obtained from the Tong Wei Hatchery (Sichuan, China). After an acclimatisation period of 4 weeks to laboratory conditions, 1200 carp, with a mean initial weight of 6·33 (SE 0·03) g, were randomly distributed into twenty-four glass aquaria (90 × 30 × 40 cm³), resulting in fifty juveniles in each aquarium. Each experimental diet was randomly assigned to aquaria in quadruplicate. Fish were fed with their respective diets to apparent satiation six times per d for the first 4 weeks and four times per d from the fifth to the ninth week. Uneaten feed was removed by siphoning at 30 min after feeding, dried and weighted to measure feed intake (FI). Water quality, closed water recirculating and oxygen auto-supplemented system were maintained as previously described by our laboratory[51]. Briefly, the water flow rate in each aquarium was maintained at 1·2 litres/min; water was drained through biofilters to remove solid substances and reduce ammonia concentration. Water temperature, pH and dissolved oxygen were 26 ± 1°C, 7·0 ± 0·3 and 5·0 (SD 0·3) mg/l, respectively. The experimental units were maintained under a natural light and dark cycle.

Table 1. Composition (g/kg dry diet) of experimental diets used for determining the effects of dietary arginine on the growth and biochemical activities of Jian carp (*Cyprinus carpio* var. Jian)

	Diets					
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fishmeal	100	100	100	100	100	100
Rice gluten meal	162	162	162	162	162	162
Amino acid mix*	135·6	135·6	135·6	135·6	135·6	135·6
α-Starch	320	320	320	320	320	320
Maize starch	153·3	155·4	157·6	159·7	161·9	161·0
Fish oil	21·8	21·8	21·8	21·8	21·8	21·8
Soya bean oil	8	8	8	8	8	8
Mineral premix†	31·6	31·6	31·6	31·6	31·6	31·6
Vitamin premix‡	11·3	11·3	11·3	11·3	11·3	11·3
Ethoxyquin	0·5	0·5	0·5	0·5	0·5	0·5
α-Cellulose	30	30	30	30	30	30
L-Arg-HCL	0	3·6	7·3	10·9	14·5	18·2
L-Gly	25·9	20·2	14·3	8·6	2·8	0
Nutrient content§						
Calculated crude protein	340·0	340·0	340·0	340·0	340·0	340·0
Analysed crude protein	330·0	330·0	330·0	330·0	330·0	330·0
Gross energy (kJ/g)	16·5	16·5	16·5	16·5	16·5	16·5
Arg	9·8	12·7	16·1	18·5	21·9	24·5

* Amino acid mix: lysine, 15·060 g; methionine, 8·265 g; threonine, 11·584 g; tryptophan, 1·523 g; histidine, 3·240 g; isoleucine, 1·362 g; phenylalanine, 7·232 g; valine, 1·305 g; glycine, 86·028 g.
† Mineral mixture (g/kg mixture): $FeSO_4 \cdot 7H_2O$, 45·767 g; $CuSO_4 \cdot 5H_2O$, 1·201 g; $ZnSO_4 \cdot 7H_2O$, 14·113 g; $MnSO_4 \cdot H_2O$, 4·089 g; KI, 2·895 g; $NaSeO_3$, 2·500 g; $CaCO_3$, 929·436 g. Ca $(H_2PO_4)_2$, 21·6 g/kg dry diet.
‡ Vitamin mixture (g/kg mixture): retinyl acetate (172 mg/g), 0·800 g; cholecalciferol (12·5 mg/g), 0·480 g; D,L-α-tocopherol acetate (50 %), 20·000 g; menadione (23 %), 0·220 g; thiamine hydrochloride (90 %), 0·113 g; riboflavin (80 %), 0·625 g; pyridoxine hydrochloride (81 %), 0·749 g; cyanocobalamin (1 %), 0·100 g; niacin (99 %), 4·165 g; D-biotin (2 %), 5·000 g; meso-inositol (99 %), 52·323 g; folic acid (96 %), 0·521 g; ascorhyl acetate (93 %), 7·161 g; calcium-D-pantothenate (90 %), 2·558 g. choline chloride, 1·3 g/kg dry diet.
§ Nutrient content: lysine, 20; methionine + cystine, 15; n-3 + n-6, 20; available phosphorus, 6. Gross energy was calculated on the basis of fuel values 19·14, 13·65, 24·27, 16·02, 14·81 and 37·65 kJ/g for fishmeal, rice gluten meal, amino acids, α-starch, maize starch and fat, respectively.

Sample collection and analysis

The procedures of sample collection were similar to those previously described in other studies conducted in our laboratory[57]. After 12-h fasting, fish from each aquaria were counted and weighed at the beginning and at the end of the feeding trial. At the beginning of the experiment, thirty fish from the same population used in the experiment were collected to determine the initial carcass proximate composition. At the end of the feeding trial, four fish from each aquarium were collected and frozen for estimating the final carcass proximate composition. A total of fifteen fish from each aquarium were anaesthetised in a benzocaine bath (50 mg/l), as described by Berdikova Bohne *et al.*[58], with a minor modification; then, the hepatopancreas, intestine and muscle were quickly collected and stored at −70°C until analysis. Another four fish from each aquarium were randomly collected for obtaining blood samples from the caudal vein with heparinised syringes, at 6 h after the last feeding, for plasma ammonia determination. The intestines of another four fish from each aquarium were used to measure the height of intestinal folds, according to Lin & Zhou[33]. The digesta of another three fish collected from each aquarium were sampled to determine intestinal microbial populations.

Proximate analysis of diets and whole body samples were performed according to methods of the Association of Official Analytical Chemists[59]. Muscle, intestine and hepatopancreas were homogenised in ten volumes (w/v) of ice-cold physiological saline solution and centrifuged at 6000 g for 20 min at 4°C; then, the supernatant was stored. GOT and GPT activities in muscle and hepatopancreas were determined with the method of Bergmeyer & Bernt[60,61]. Blood was centrifuged at 4000 g for 15 min; then, the supernatant fluid was collected for ammonia determination, as described by Tantikitti & Chimsung[62]. Trypsin and chymotrypsin activities were determined according to Hummel[63]. Amylase and lipase activities were measured, as described by Furné *et al.*[64]. AKP, Na^+/K^+-ATPase, γ-glutamyl transpeptidase (γ-GT) and CK activities in the intestine were determined according to Bessey *et al.*[65], McCormick[66], Bauermeister *et al.*[67] and Tanzer & Gilvarg[68], respectively. The intestinal content was extruded for estimating the counts of *Lactobacillus*, *E. coli* and *Aeromonas* using standard techniques, as described by Refstie *et al.*[69].

Analysis of target of rapamycin and eIF4E-binding protein gene expression in muscle, hepatopancreas and intestine

Total RNA was extracted from muscle, hepatopancreas, proximal-intestine (PI), mid-intestine (MI) and distal intestine (DI) using an RNAiso plus kit (Takara, Dalian, Liaoning, China). The quality of total RNA was judged by spectrophotometry at 260 and 280 nm. Subsequently, complementary DNA was synthesised using a PrimeScript™ RT reagent Kit (Takara), according to the manufacturer's instructions. Briefly, oligo dT primers (50 μM) were used to reverse transcribe respective

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Table 2. Real-time PCR primer sequences

Name	Sequences of primers	bp
TOR		
Forward	5′-ATC ATA CGC ATC CAG TCC ATT G-3′	22
Reverse	5′-GGT CAT TAG CCA GTA GAG TGT TC-3′	23
4E-BP		
Forward	5′-GCT ACC TCA CGA CTA TTG C-3′	19
Reverse	5′-TTC TTG CTT GTC ACT CCT G-3′	19
β-Actin		
Forward	5′-CGT GAT GGA CTC TGG TGA TG-3′	20
Reverse	5′-TCG GCT GTG GTG GTG AAG-3′	18

TOR, target of rapamycin; 4E-BP, eIF4E-binding protein.

RNA in the presence of PrimeScript™ RT enzyme mix I, 5 × PrimeScript™ buffer, random 6 mers (100 μM) and RNase-free distilled water at 37°C for 15 min, following inactivation at 85°C for 5 s. Specific primers for *TOR* and *4E-BP* genes were designed with Primer Premier software (Premier Biosoft International, Palo Alto, CA, USA) according to sequences of Jian carp (Genbank accession no. FJ899680 and HQ010440, respectively) cloned in our laboratory. Real-time PCR were performed for TOR and 4E-BP according to standard protocols with the primers indicated in Table 2. Briefly, complementary DNA (2 μl) was reacted with forward and reverse primers, SYBR Premix Ex Taq™ II (2 ×; 7·5 μl; Takara) and RNase-free distilled water in a 15 μl final reaction volume. PCR were performed using a Chromo 4™ continuous fluorescence detector (Bio-Rad, Hercules, CA, USA). The thermocycling conditions for TOR and 4E-BP were the following: forty cycles at 95°C for 10 s, 95°C for 5 s, 60°C for 53 s and 95°C for 10 s, 95°C for 5 s, 59·5°C for 30 s, respectively. The expression levels of the *TOR* and *4E-BP* genes were normalised to the expression levels of a housekeeping common carp gene, *β-actin*. Each assay was performed with five replications. The concentration of the target gene was calculated based on the threshold cycle number (cycle threshold). The cycle threshold for each sample was determined by using MJ Opticon Monitor Software (version 3.1; Bio-Rad, Hemel Hempstead, Herts, UK). In addition, the complementary DNA concentration in each sample was determined according to gene-specific standard curves. Standard curves were generated for both target and endogenous control genes based on 10-fold serial dilutions. All standard curves exhibited correlation coefficients higher than 0·99, and the corresponding real-time PCR efficiencies ranged between 0·90 and 1·10.

Calculations and statistical analysis

Data on initial body weight, final body weight, FI, proximate composition of feed and carcass, hepatopancreas and intestine weight, intestine and body length, and hepatopancreas and intestine protein were used to calculate the following parameters:

Feed efficiency (FE) = (g weight gain/g FI) × 100;

Specific growth rate = ((ln final weight − ln initial weight)/ number of d) × 100;

Protein efficiency ratio = g weight gain/g protein intake;

Protein retention value (PRV) = (final total body protein − initial body protein)/total protein intake;

Ash retention value = g fish ash gain/g ash intake;

Intestosomatic index (ISI) = (g wet intestine weight/g wet body weight) × 100;

Hepatosomatic index = (g wet hepatopancreas weight/g wet body weight) × 100;

Relative gut length (RGL) = digestive tract length (cm)/total body length (cm);

Intestine protein content = (g intestine protein/g wet intestine weight) × 100;

Hepatopancreas protein content = (g hepatopancreas protein/g wet hepatopancreas weight) × 100;

All data were subjected to a one-way ANOVA. Differences between the treatment mean values were determined using a Duncan's multiple-range test at a $P < 0.05$ level of significance. A quadratic regression model was used to determine the optimal level of dietary arginine.

Results

Growth performance

Dietary arginine did not have a significant effect on the survival rate (>97%) of juvenile Jian carp. No pathological signs were observed during the trial. As shown in Table 3, the lowest specific growth rate (SGR) was found in fish fed with the basal diet ($P<0.05$). FI significantly increased with higher levels of dietary arginine up to 16·1 g arginine/kg diet and decreased thereafter ($P < 0.05$; Table 3). Quadratic regression analysis showed that SGR and FI increased with increasing levels of dietary arginine. The following equations were obtained for SGR and for FI, respectively: $Y = 2·531 + 0·0862x − 0·0024x^2$, R^2 0·930, $P<0.05$ and $Y = 35·256 + 2·5749x − 0·0762x^2$, R^2 0·942, $P<0.05$. On the basis of the aforementioned quadratic regression equation, the arginine requirement for the juvenile carp was estimated to be 18·0 g/kg diet, corresponding to 55·0 g/kg dietary protein (Fig. 1). Values of FE, protein efficiency ratio and PRV were the highest for fish fed with diets containing 18·5 g arginine/ kg diet and the lowest for fish fed with diets containing 12·7 g arginine/kg diet ($P<0.05$). The ash retention value increased with dietary arginine levels up to 18·5 g/kg diet ($P<0.05$), whereas higher arginine levels resulted in a plateau-like response ($P>0.05$). The following equations were obtained for PRV and for ash retention value, respectively: $Y = 26·646 + 0·7721x − 0·0198x^2$, R^2 0·699 and $Y = 30·496 + 0·5574x − 0·0129x^2$, R^2 0·738.

Glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities in muscle and hepatopancreas

GOT and GPT activities in muscle and hepatopancreas, as well as plasma ammonia content (PAC) are given in Table 4. GOT activities in muscle and hepatopancreas were the highest for fish fed with diets containing 18·5 g arginine/ kg diet and the lowest for fish fed with the basal diet

Table 3. Growth, feed intake (FI) and conversion efficiency of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed with diets containing graded levels of dietary arginine

(Mean values with their standard errors for four replicates)

Dietary Arg levels (g/kg)...	9·8		12·7		16·1		18·5		21·9		24·5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
IBW (g)	6·34	0·017	6·34	0·009	6·35	0·023	6·33	0·008	6·31	0·009	6·34	0·004
FBW (g)	46·2[a]	0·47	47·7[a]	0·98	50·7[b]	0·72	50·8[b]	0·52	48·7[a,b]	0·85	47·0[a]	1·37
SGR (%/d)	3·155[a]	0·016	3·203[a]	0·033	3·297[b]	0·020	3·304[b]	0·017	3·243[a,b]	0·028	3·179[a]	0·046
FI (g)	52·9[a]	0·21	56·0[b]	0·63	57·5[c]	0·43	56·1[b]	0·33	55·0[b]	0·48	52·8[a]	0·36
FE	75·4[a,b]	0·65	73·9[a]	0·97	77·2[a,b]	0·67	79·3[b]	0·72	77·2[a,b]	1·17	77·0[a,b]	2·18
PER	2·298[a,b]	0·020	2·252[a]	0·030	2·352[a,b]	0·020	2·416[b]	0·022	2·353[a,b]	0·036	2·348[a,b]	0·067
PRV	32·7[a]	0·28	32·5[a]	0·42	34·0[a,b]	0·30	34·8[b]	0·31	33·8[a,b]	0·51	33·7[a,b]	0·95
ARV	35·2[a]	0·31	35·2[a]	0·47	36·1[a,b]	0·33	37·6[b]	0·34	36·7[a,b]	0·56	36·8[a,b]	1·06

IBW, initial body weight; FBW, final body weight; SGR, specific growth rate; FE, feed efficiency; PER, protein efficiency ratio; PRV, protein retention value; ARV, ash retention value.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

($P<0.05$). In addition, GOT activity in muscle showed a quadratic response to increasing dietary arginine concentrations ($Y = -1119.5 + 431.29x - 11.778x^2$, R^2 0.882, $P<0.05$). GPT activities in muscle and hepatopancreas were the highest for fish fed with diets containing 16·1 g arginine/kg diet ($P<0.05$). PAC was the lowest for fish fed with diets containing 18·5 g arginine/kg diet ($P<0.05$).

Hepatopancreas and intestine growth and development

As shown in Table 5, the hepatopancreas weight was the lowest for fish fed with the basal diet, followed by 24·5 and 21·9 g arginine/kg diet, and it was the highest for fish fed with diets containing 12·7 g arginine/kg diet ($P<0.05$). The hepatosomatic index and protein content were the highest for fish fed with diets containing 12·7 and 18·5 g arginine/kg diet ($P<0.05$), respectively. The following equations were obtained for hepatopancreas weight and for hepatopancreas protein content, respectively: $Y = 0.4010 + 0.1535x - 0.0045x^2$, R^2 0.756 and $Y = 0.7226 + 0.0325x - 0.0007x^2$, R^2 0.810. Intestine length significantly increased with increasing dietary arginine levels up to 12·7 g arginine/kg diet ($P<0.05$), and there were no differences between 12·7 and 21·9 g arginine/ kg diet levels ($P > 0.05$). The RGL showed a non-significant tendency towards an improvement of dietary arginine levels above 12·7 g arginine/kg diet ($P>0.05$), with the only exception of fish fed with 18·5 g arginine/kg diet. Similar patterns were found for intestine weight. The ISI was the highest for fish fed with the basal diet and the lowest for fish fed with a diet containing 21·9 g arginine/kg diet ($P<0.05$). The intestine protein content was the highest for fish fed with a diet containing 16·1 g arginine/kg diet ($P<0.05$) and the lowest for fish fed with the basal diet. Quadratic regression analysis showed that intestine length, RGL, intestine weight and ISI increased or decreased with higher levels of dietary arginine. The following equations were obtained for intestine length, RGL, intestine weight and ISI, respectively: $Y = 6.0540 + 1.6892x - 0.0482x^2$, R^2 0.811; $Y = 103.15 + 6.6738x - 0.1953x^2$, R^2 0.777; $Y = 0.7446 + 0.1236x - 0.0037x^2$, R^2 0.757; and $Y = 4.8646 - 0.1580x + 0.0037x^2$, R^2 0.9369, $P<0.05$.

Hepatopancreas and intestine enzyme activities

The trypsin activity in the intestine was compared across dietary treatments (Table 6). The activity was responsive to dietary arginine by increasing with graded levels of arginine up to 16·1 g/kg diet ($P<0.05$), and there was no difference between 16·1 and 18·5 g/kg diet ($P>0.05$), and was positively related to the activity of the hepatopancreas ($r + 0.939$, $P<0.01$). Similarly, the chymotrypsin activity in the hepatopancreas was the highest for fish fed with a diet containing 16·1 g arginine/kg diet and the lowest for fish fed with the diet containing 24·5 g arginine/kg diet ($P<0.05$; Table 6). The chymotrypsin activity in the intestine was the highest for fish fed with diets containing 18·5 g arginine/kg diet ($P<0.05$). Lipase activities in hepatopancreas showed a non-significant tendency towards the improvement of dietary arginine levels ($P>0.05$), with the only exception for fish fed with 24·5 g arginine/kg diet, exhibiting significantly lower values ($P<0.05$). In the intestine, lipase activities increased with higher levels of dietary arginine up to 18·5 g arginine/kg diet ($P<0.05$) and decreased thereafter. The following equations were obtained for trypsin activities and for lipase activities, respectively, in the hepatopancreas: $Y = 1.4137 + 0.2803x - 0.0081x^2$, R^2 0.8836, $P<0.05$ and $Y = 1098.3 + 98.428x - 3.0784x^2$, R^2 0.858, $P=0.05$. Amylase activities in the hepatopancreas increased with higher levels of dietary arginine up to
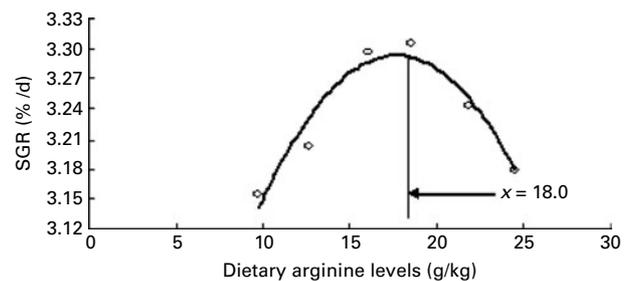
Fig. 1. Quadratic regression analysis of specific growth rate (SGR, %/d) according to dietary arginine levels ($y = -0.0024x^2 + 0.0862x + 2.531$, R^2 0.930). Each point represents the mean of four groups of Jian carp with fifty fish per group. Arginine requirement estimated from SGR was 18·0 g/kg diet.

Table 4. Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities in muscle and hepatopancreas; plasma ammonia content of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed with diets containing graded levels of arginine

(Mean values with their standard errors for four replicates)

Dietary Arg levels (g/kg)…	9·8		12·7		16·1		18·5		21·9		24·5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Glu-oxaloacetate transaminase activities (U/g tissue)												
Muscle	2015[a]	42·9	2407[b,c]	81·7	2657[c]	75·2	3032[d]	102	2586[b,c]	109	2390[b]	71·3
Hepatopancreas	1766[a]	66·3	1980[b,c]	60·2	2033[c]	55·3	2639[d]	35·7	1997[c]	45·1	1819[a,b]	67·7
Glu-pyruvate transaminase activities (U/g tissue)												
Muscle	455[a]	9·31	522[b,c]	18·0	612[d]	18·2	552[c]	7·46	514[b,c]	16·3	492[a,b]	9·95
Hepatopancreas	574[b]	9·51	604[b,c]	11·7	642[c]	21·8	612[b,c]	18·2	567[b]	18·0	507[a]	17·9
Ammonia content (μmol/l)												
Plasma	233[d]	4·80	122[b]	3·92	110[a,b]	4·80	102[a]	7·33	188[c]	4·80	192[c]	7·33

[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

16·1 g arginine/kg diet ($P<0.05$). No significant differences were found in intestinal amylase activities between dietary treatments ($P>0.05$).

As shown in Table 6, folds height in the PI was the highest for fish fed with a diet containing 18·5 g arginine/kg diet ($P<0.05$); the height values decreased with diets containing 16·1, 21·9, 12·7 and 24·5 g arginine/kg diet; finally, the lowest value occurred in fish fed with the basal diet ($P<0.05$). The highest folds height in MI and DI were obtained for fish fed with a diet containing 16·1 g arginine/kg diet ($P<0.05$). In addition, Na$^+$/K$^+$-ATPase activity in the PI and DI increased with higher levels of dietary arginine up to 16·1 g arginine/kg diet, and the lowest activities occurred in fish fed with a diet containing 24·5 g arginine/kg diet (Table 6). In the MI, the Na$^+$/K$^+$-ATPase activity was the highest for fish fed with a diet containing 18·5 g arginine/kg diet ($P<0.05$). In the PI, the Na$^+$/K$^+$-ATPase activity showed a quadratic response to increasing dietary arginine concentrations ($Y = -778.33 + 121.28x - 3.551x^2$, R^2 0·953, $P<0.05$). Intestinal AKP activities increased as dietary arginine levels rose up to 16·1 g arginine/kg diet ($P<0.05$). SGR was positively related to AKP activities in the PI ($r + 0.921$, $P<0.05$). The following equations were obtained for AKP activities in the PI and MI, respectively: $Y = -39.976 + 7.6209x - 0.2240x^2$, R^2 0·998, $P<0.05$ and $Y = -33.781 + 6.5985x - 0.1914x^2$, R^2 0·920, $P<0.05$.

Similar patterns were found in intestinal γ-GT activity. The following equations were obtained for γ-GT activities in the PI, MI and DI, respectively: $Y = -10.901 + 2.800x - 0.0833x^2$, R^2 0·944, $P<0.05$; $Y = -4.897 + 1.5857x - 0.0450x^2$, R^2 0·973, $P<0.01$; and $Y = -14.819 + 4.3235x - 0.1299x^2$, R^2 0·884, $P<0.05$. CK activities in the whole intestine significantly increased up to 18·5 g arginine/kg diet and showed quadratic responses to increasing levels of dietary arginine ($Y = -487.81 + 91.386x - 2.5196x^2$, R^2 0·855).

Intestinal microflora population

As shown in Table 7, *Aeromonas* and *E. coli* were the lowest for fish fed with a diet containing 16·1 g arginine/kg diet and the highest for fish fed with the basal diet ($P<0.05$). *Lactobacillus* populations significantly increased with higher levels of dietary arginine up to 16·1 g/kg diet ($P<0.05$), and there were no differences between the 16·1 and 21·9 g/kg diet levels ($P>0.05$). Quadratic regression analysis showed that the populations of intestinal microbiota increased or decreased with higher levels of dietary arginine. The following equations were obtained for *Aeromonas*, *E. coli* and *Lactobacillus*, respectively: $Y = 10.206 - 0.2213x + 0.0063x^2$, R^2 0·992, $P<0.01$; $Y = 10.343 - 0.3479x + 0.0100x^2$, R^2 0·938, $P<0.05$; and $Y = 1.6435 + 0.5783x - 0.0150x^2$, R^2 0·972, $P<0.01$.

Table 5. Hepatopancreas and intestinal activities of Jian carp (*Cyprinus carpio* var. Jian) fed with diets containing graded levels of dietary arginine

(Mean values with their standard errors for four replicates)

Dietary Arg levels (g/kg)…	9·8		12·7		16·1		18·5		21·9		24·5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Hepatopancreas												
Weight (g)	1·42[a]	0·08	1·74[c]	0·05	1·68[c]	0·06	1·68[c]	0·04	1·60[b,c]	0·06	1·50[a,b]	0·05
Index	3·33[a,b]	0·13	3·46[b]	0·15	3·01[a]	0·12	3·18[a,b]	0·10	3·11[a,b]	0·16	3·04[a]	0·13
Protein content	0·98[a]	0·04	1·04[a,b,c]	0·04	1·03[a,b]	0·03	1·14[c]	0·04	1·12[b,c]	0·02	1·12[b,c]	0·03
Intestine												
Length (cm)	17·5[a]	0·35	20·5[b,c]	0·68	21·1[b,c]	0·63	20·2[b,c]	0·53	19·6[b,c]	0·38	18·9[b]	0·37
Relative length	148[a]	3·61	160[a,b]	4·73	158[a,b]	4·51	161[b]	3·99	153[a,b]	3·06	151[a,b]	2·76
Weight (g)	1·57[a]	0·07	1·75[b,c]	0·04	1·83[c]	0·04	1·73[b,c]	0·06	1·61[a,b]	0·04	1·60[a,b]	0·03
Index	3·66[c]	0·09	3·48[b,c]	0·14	3·27[a,b]	0·09	3·27[a,b]	0·13	3·11[a]	0·11	3·27[a,b]	0·14
Protein content	1·06[a]	0·03	1·15[a,b]	0·05	1·19[b]	0·05	1·17[a,b]	0·03	1·08[a,b]	0·04	1·13[a,b]	0·04

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

Table 6. Enzymatic activities in hepatopancreas and intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed with diets containing graded levels of dietary arginine

(Mean values with their standard errors for four replicates)

Dietary Arg levels (g/kg)...	9·8		12·7		16·1		18·5		21·9		24·5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Enzymatic activities in hepatopancreas (U/g tissue)												
Trypsin	0·53[a]	0·02	0·89[c]	0·03	1·00[d]	0·02	1·07[d]	0·03	0·74[b]	0·02	0·68[b]	0·01
Chymotrypsin	4·72[a]	0·20	4·75[a]	0·15	6·11[c]	0·17	5·54[b]	0·21	5·00[a]	0·06	4·67[a]	0·20
Lipase	1783[b]	41·9	1837[b]	34·2	1838[b]	34·2	1919[b]	27·0	1784[b]	41·9	1648[a]	65·1
Amylase	1113[a]	23·0	1242[b,c]	34·6	1275[c]	15·8	1271[c]	31·2	1263[a,b]	30·8	1200[b,c]	28·9
Enzymatic activities in intestine (U/g tissue)												
Trypsin	1·29[a]	0·07	1·47[b]	0·05	1·72[c]	0·05	1·72[c]	0·07	1·35[a,b]	0·05	1·40[a,b]	0·04
Chymotrypsin	2·53[a]	0·11	2·86[a,b]	0·10	2·86[a,b]	0·11	4·31[c]	0·16	2·92[b]	0·06	2·53[a]	0·07
Lipase	1513[a]	68·4	1594[a,b,c]	49·8	1730[b,c]	54·0	1757[c]	49·8	1648[a,b,c]	49·8	1567[a,b]	54·0
Amylase	1196	24·5	1225	23·3	1246	10·0	1242	33·3	1238	23·0	1204	52·2
Folds height (μm)												
Proximal	439[a]	2·51	470[c]	3·35	488[d]	2·46	491[d]	4·78	478[c]	3·27	459[b]	3·73
Mid	282[a]	4·77	298[b]	3·00	309[c]	3·17	289[a,b]	3·45	285[a]	3·24	287[a]	3·07
Distal	265[a]	1·70	283[b]	4·39	294[c]	2·96	286[b,c]	3·72	285[b,c]	3·42	269[a]	1·78
Na+, K+-ATPase activities in intestine (μmol P released g/tissue per h)												
Proximal	83·6[b]	2·14	157[c]	5·62	267[d]	6·09	265[d]	5·71	163[c]	6·09	63·2[a]	2·04
Mid	115[a]	1·58	124[a]	4·04	209[c]	7·38	252[d]	6·72	184[b]	7·18	182[b]	6·20
Distal	124[b,c]	4·53	132[c]	4·64	166[d]	6·36	174[d]	6·28	113[a]	2·65	105[a]	4·03
Alkaline phosphatase activities in intestine (mmol nitrophenol released g/tissue per h)												
Proximal	13·2[a]	0·31	20·8[c]	0·40	24·2[d]	0·54	24·7[d]	0·33	19·5[c]	0·56	12·2[a]	0·48
Mid	13·7[a]	0·42	16·7[b]	0·71	23·5[d]	0·43	23·8[d]	0·60	18·8[c]	0·48	12·7[a]	0·49
Distal	5·67[a]	0·21	7·50[b]	0·22	7·83[c]	0·17	9·83[c]	0·31	7·33[b]	0·21	7·50[b]	0·22
γ-Glutamyl transpeptidase activities in intestine (mmol 5-amino-2-nitrobenzoate released g/tissue per min)												
Proximal	8·63[b,c]	0·15	10·9[c]	0·33	12·8[d]	0·19	12·9[d]	0·41	9·67[b]	0·29	8·07[a]	0·08
Mid	6·28[a]	0·17	8·07[c]	0·29	8·79[d]	0·10	9·28[d]	0·30	8·00[c]	0·27	7·02[b]	0·19
Distal	15·5[a,b]	0·45	18·0[c]	0·46	21·6[d]	1·16	22·0[d]	0·71	15·9[b]	0·53	13·8[a]	0·34
Creatine kinase activities (μmol P released g/tissue per h)												
Intestine	183[a]	4·30	234[b]	2·44	322[d]	7·02	383[e]	8·46	284[c]	4·63	241[b]	3·75

[a,b,c,d,e] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

Relative expression of target of rapamycin and eIF4E-binding protein in muscle, hepatopancreas and intestine

TOR mRNA levels were the highest for fish fed with a diet containing 16·1 g arginine/kg diet in muscle and hepatopancreas ($P<0.05$; Fig. 2). Patterns of TOR mRNA in the PI and MI were opposite compared with those in the muscle and hepatopancreas (Fig. 2). TOR mRNA levels in the DI increased with higher levels of dietary arginine up to 12·7 g/kg diet (Fig. 2; $P<0.05$), whereas no significant differences were found with a further increase in dietary arginine concentration ($P>0.05$). In the hepatopancreas, 4E-BP mRNA levels were the lowest for fish fed with a diet containing 16·1 g arginine/kg diet and the highest for fish fed with a diet containing 24·5 g arginine/kg diet (Fig. 3; $P<0.05$). The levels of 4E-BP

mRNA in the PI slightly increased with dietary arginine levels up to 16·1 g/kg diet (Fig. 3; $P>0.05$); the highest values were obtained in fish fed with a diet containing 18·5 g arginine/kg diet and then decreased. In the MI, 4E-BP mRNA levels significantly increased with dietary arginine levels up to 12·7 g/kg diet (Fig. 3; $P<0.05$), and there were no differences between 16·1 and 24·5 g arginine/kg diet levels ($P>0.05$). The levels of 4E-BP mRNA in muscle significantly decreased with dietary arginine levels up to 12·7 g/kg diet (Fig. 4; $P<0.05$); then, the levels remained approximately constant. In the DI, 4E-BP mRNA levels (Fig. 4) were the highest for fish fed with a diet containing 24·5 g arginine/kg diet ($P<0.05$) and the lowest for fish fed with a diet containing 16·1 g arginine/kg diet ($P<0.05$). The following equations were obtained for 4E-BP mRNA levels in muscle and in DI,

Table 7. Intestine flora of Jian carp (*Cyprinus carpio* var. Jian) fed diets containing graded levels of dietary arginine

(Mean values with their standard errors for four replicates)

Dietary Arg levels (g/kg)...	9·8		12·7		16·1		18·5		21·9		24·5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Intestine flora (log CFU/g intestine content)												
Aeromonas hydrophila	8·65[b]	0·01	8·40[a]	0·05	8·26[a]	0·03	8·29[a]	0·05	8·38[a]	0·02	8·56[b]	0·10
Escherichia coli	7·96[d]	0·02	7·45[a,b]	0·02	7·31[a]	0·06	7·41[a,b]	0·07	7·55[b]	0·03	7·80[c]	0·05
Lactobacillus	5·90[a]	0·11	6·48[b]	0·01	7·22[d]	0·15	7·14[d]	0·01	7·12[d]	0·05	6·84[c]	0·09

CFU, colony-forming units.
[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
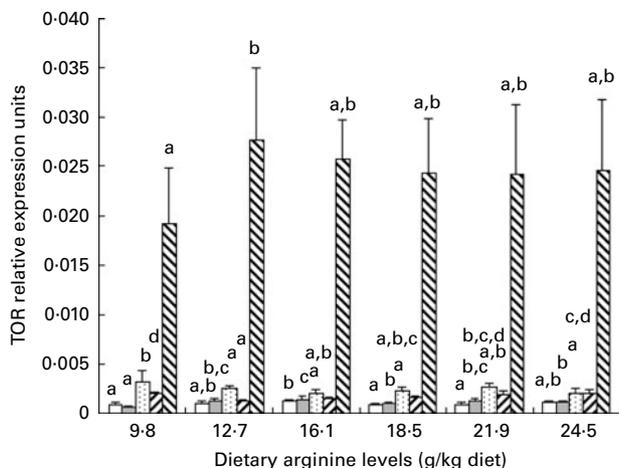
Fig. 2. Relative expression of target of rapamycin (TOR) mRNA in juvenile Jian carp (*Cyprinus carpio* var. Jian) fed with diets containing graded levels of arginine. Values are means for five fish per treatment, with standard deviations represented by vertical bars (n 5). [a,b,c,d] Mean values with unlike letters were significantly different ($P < 0.05$). □, Muscle; ▩, hepatopancreas; ▨, proximal intestine; ▨, mid intestine; ▨, distal intestine.

respectively: $Y = 0.001 - 9E - 05x + 2E - 06x^2$, R^2 0.955, $P < 0.05$ and $Y = 0.0013 - 0.0001x + 4E - 06x^2$, R^2 0.906, $P < 0.05$.

Discussion

The importance of dietary arginine for normal growth of Jian carp was demonstrated in the present study. SGR increased with higher dietary arginine concentrations up to an optimum level. Similar observations have been reported in Indian major carp[4,70,71], black sea bream (*Sparus macrocephalus*)[72], rainbow trout[73] and channel catfish (*Ictalurus punctatus*)[1]. In the present study, FI and FE increased with higher arginine levels up to a level similar to those found in other fish
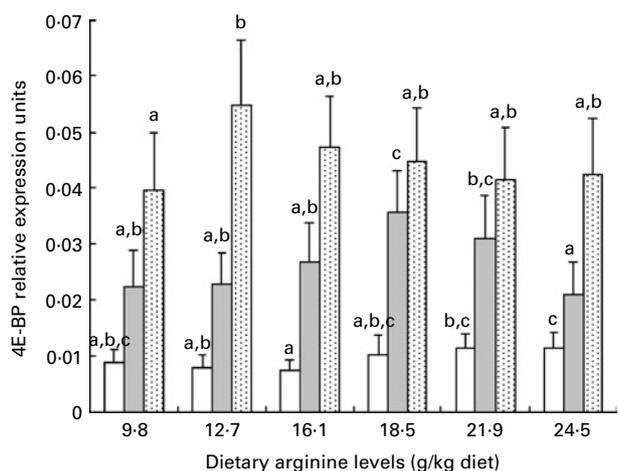
Fig. 3. Relative expression of eIF4E-binding protein (4E-BP) mRNA in hepatopancreas, proximal and mid-intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed with diets containing graded levels of arginine. Values are means for five fish per treatment, with standard deviations represented by vertical bars. [a,b,c] Mean values with unlike letters were significantly different ($P < 0.05$). □, Hepatopancreas; ▩, proximal intestine; ▨, mid intestine.
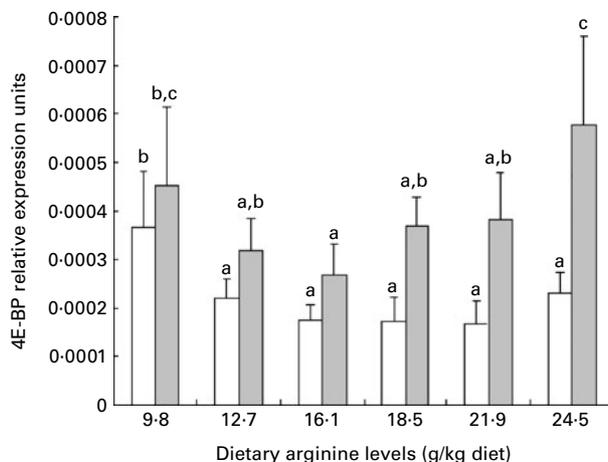
Fig. 4. Relative expression of eIF4E-binding protein (4E-BP) mRNA in muscle and distal intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed with diets containing graded levels of arginine. Values are means for five fish per treatment, with standard deviations represented by vertical bars. [a,b,c] Mean values with unlike letters were significantly different ($P < 0.05$). □, Muscle; ▩, distal intestine.

species[4,71]. SGR was positively related to FI and FE (r $+0.848$, $P < 0.05$; r $+0.845$, $P < 0.05$). This result indicates that the enhancement of fish growth was partly attributed to the increment in FI and FE. Fish growth mainly involves protein retention in muscle, epithelial and connective tissue[74]. A continuous supply of amino acids is required for protein synthesis because proteins are continually used for animal growth and tissue repair[75]. In the present study, PRV significantly increased with higher levels of dietary arginine up to an optimum arginine level that supported the highest SGR. Besides protein synthesis, the improvement of fish growth with arginine supplementation might be due to its role as a secretagogue of growth-regulating hormones[76]. Fish fed with diets containing arginine above the optimum level did not exhibit additional growth. Similar results have been obtained in a few studies in rainbow trout[77] and Nile tilapia (*Oreochromis niloticus*)[78], whereas such results were not observed in other species such as carp[79], sea bass[2] and channel catfish[80]. The reduction in weight gain with arginine levels above the requirement level might be due to (1) extra energy expenditure for deamination; (2) disturbance of absorption and utilisation of other amino acids; (3) lower palatability of the diet; or (4) toxic effects and stress[81]. A reduction of FI was regarded as the primary factor responsible for the depressed growth observed in Atlantic salmon fry[82] and European sea bass[83]. The arginine requirement estimated from SGR by using a quadratic regression analysis was 18.0 g/kg diet, which corresponded to 55.0 g/kg of dietary protein (Fig. 1). This value was higher than that of channel catfish with 33–38 g/kg of dietary protein[84] and Japanese flounder (*Paralichthys olivaceus*) with 41.4 g/kg of dietary protein[85] and lower than that of black sea bream with 77.4–81.3 g/kg of dietary protein[72].

Protein deposition was mainly associated with amino acid metabolism in fish[5]. Unbalanced dietary amino acid influenced ammonia formation and decreased amino acid

utilisation and protein retention[86]. In the present study, the PAC was lower for fish fed with optimum dietary arginine levels, supporting a higher protein efficiency ratio in this group. Therefore, amino acids were available in an appropriate balance for body protein synthesis with the optimal arginine level. Higher PAC was found in Jian carp fed with a moderate excess of arginine than those fed with the optimal level. Similarly, Tulli et al.[9] observed that there was an increase in plasma ammonia nitrogen concentration in European sea bass fed with plant protein diets under a moderate or large excess of dietary arginine. This higher PAC might be the result of amino acid imbalance and/or catabolism of excessive arginine[77,85]. This scenario might explain the poor growth performance in fish fed with diets containing 21·9 and 24·5 g arginine/kg diet. Moreover, Gouillou-Coustans et al.[87] showed that the plasma urea concentration was responsive to arginine intake in turbot (Psetta maxima). Hence, a more extensive study is necessary to investigate the effects of arginine on nitrogen excretion.

Pelletier et al.[88] found that amino acid metabolism correlated with growth rates in Atlantic cod (Gadus morhua). Moreover, GOT and GPT are considered to be the most important amino acid catabolism enzymes of teleostean fish[6]. In the present study, GOT and GPT activities in muscle and hepatopancreas significantly increased with higher dietary arginine concentrations until a certain point; then, the activities decreased with further increases in dietary arginine levels, supporting the PRV results. Similar observations were reported for juvenile Jian carp supplementation with methionine hydroxy analogue in practical diets[10]. The present results indicate an efficient use of dietary amino acids for growth when fish are fed with an optimal dietary arginine concentration.

Fish growth is mainly associated with digestive and absorptive ability[11,12]. Digestion and absorption of nutrients depend on the activity of digestive enzymes and brush-border membrane enzymes, which are responsible for breaking down and assimilating food[18]. Fish exocrine pancreas synthesises and secretes a large number of digestive enzymes into the intestinal lumen, such as trypsin, chymotrypsin, lipase and amylase[19,20]. The potential energy of the Na gradient created by the Na$^+$/K$^+$-ATPase is used by many transport systems to move, for example, phosphate, amino acids or glucose into the cells[89]. AKP, an important enzyme in the absorptive process in fish, is considered to be a general marker of nutrient absorption[90], and γ-GT is involved in peptide transport[91]. CK has a key role in the energy metabolism of cells, because it catalyses the transfer of phosphate to creatine in an ATP-dependent manner[92]. In the present study, trypsin, chymotrypsin and amylase activities in hepatopancreas significantly increased with higher levels of dietary arginine. Similarly, activities of Na$^+$/K$^+$-ATPase, AKP, γ-GT and CK in the intestine significantly increased with dietary arginine levels. In addition, SGR was positively related to the activity of these enzymes ($r_{trypsin}$ +0·895, $P<0.05$; $r_{chymotrypsin}$ +0·889, $P<0.05$; $r_{amylase}$ +0·854, $P<0.05$; $r_{Na^+/K^+-ATPase}$ +0·957, $P<0.01$; r_{AKP} +0·921, $P<0.01$; $r_{γ-GT}$ +0·877, $P<0.05$). These results demonstrate that the higher growth performance in fish fed with optimal arginine levels was related to a higher activity of enzymes involved in digestion and absorption. Furthermore, studies from our laboratory found that glutamine[33] and lysine[34] improved digestive and absorptive enzyme activities in juvenile Jian carp. To date, information regarding the effect of arginine on the activity of digestion and absorption enzyme is scarce. Evidence from a structural analysis has shown that arginine residues have an important role in digestion and absorption enzymes[28,29,31,32]. In addition, arginine metabolites, such as polyamines and NO, might be involved in the beneficial effects on digestive and absorptive enzyme activities. Péres et al.[93] showed that supplementing spermine to microparticulate diets increased pancreatic enzyme activities in sea bass larvae. Fish exocrine pancreas is the main site for digestive enzyme synthesis and secretion[19,20]. Studies on mice and pigs indicated that NO has an active role in pancreatic secretion[24,25]. Moreover, the enhancement of digestive and absorptive enzyme activities with arginine might be related to the integrity, growth and development of fish digestive organs, which are the foundation of digestion and absorption. Lovett & Felder[94] reported that the activity of the digestive enzymes was correlated with the growth and development of the hepatopancreas in white shrimp (Penaeus setiferus). In the present study, the hepatopancreas weight and protein content showed a similar trend with the digestive enzyme activities, suggesting a beneficial effect of arginine on hepatopancreas growth and development. The hepatosomatic index of sea bass was increased significantly up to a point and decreased thereafter as dietary arginine levels increased[2]. Intestine length, weight and protein content increased with increasing dietary arginine concentrations, suggesting that arginine also stimulated fish intestinal growth and development. Furthermore, folds height was responsive to dietary arginine in the present study, which indicates the improvement of intestinal morphometric integrity. The beneficial effect of arginine on the integrity, growth and development of fish hepatopancreas and intestine might be related to polyamines. Polyamines (putrescine, spermidine and spermine), important products of arginine degradation in cells, are essential for cell proliferation and differentiation[95]. Like other intestinal mucosal cells[96], fish brush-border membrane might depend on polyamines for proliferation and differentiation. However, more studies are required to elucidate a more detailed mode in which arginine mediates the digestive and absorptive ability in fish.

Intestinal microbiota has an important role in fish health status[36], and alterations in microbial balance might result in detrimental effects to hosts[37]. In the present study, Aeromonas and E. coli gradually decreased with dietary arginine levels, whereas Lactobacillus gradually increased. Although limited information is available regarding the effects of arginine on fish intestinal microbial populations, similar observations were reported in juvenile Jian carp supplemented with methionine[35]. The underlying mechanism needs to be further investigated.

In the present study, the hepatopancreas and intestine protein content increased with increasing dietary arginine,

suggesting the improvement of protein synthesis. Translation initiation, the limiting step in protein synthesis, is regulated by the TOR signalling pathway[41]. A study from our laboratory indicated that TOR was involved in the regulation of fish IEC protein synthesis with Gln supplementation (J Jiang and XQ Zhou, unpublished results). Fish growth consists primarily of an increase in body muscle mass by protein synthesis and accretion[97]. Seiliez *et al*.[42] showed that re-feeding induces the activation of the TOR pathway in rainbow trout muscle by enhancing the phosphorylation of TOR and 4E-BP1. In liver, a protein anabolic response was accompanied by increased phosphorylation of 4E-BP1 in human and rats after a protein meal[98–100] and elevated phosphorylation of TOR in rainbow trout[43]. Moreover, arginine regulated 4E-BP1 phosphorylation through the mTOR signalling pathway in IEC6 and in IEC18 rat intestinal epithelial[15] and intestinal porcine epithelial cell -1[16]. These studies indicate a stimulation of an amino acid-sensitive target of a rapamycin signalling pathway involved in regulating protein accretion in mammals and fish. To our knowledge, the present study is the first to determine the effect of dietary arginine on the mRNA expression of major kinases involved in the TOR pathway in a fish species. Extending these observations, we reported here that patterns of difference in mRNA levels of 4E-BP, the inhibitor of translation, were properly opposite to TOR mRNA levels in the hepatopancreas, muscle and intestine, suggesting that arginine might decrease the inhibition of translation and increase TOR activity, thus improving the synthesis of proteins. These results suggest that arginine might improve protein synthesis in fish through the TOR pathway. These novel findings might explain our observation that arginine enhanced fish protein retention, intestinal enzyme activities and hepatopancreatic and intestinal growth. It is worth noting, however, that patterns of difference in TOR and 4E-BP mRNA levels in the PI and MI were opposite to that in hepatopancreas, muscle and DI. Understanding the underlying mechanisms require further studies.

Therefore, we conclude that arginine could improve fish growth and intestinal enzyme activities and maintain an intestinal microbial balance by promoting the growth of health-promoting bacteria and decreasing the growth of harmful bacteria in juvenile Jian carp. The arginine requirement of Jian carp was estimated by using a quadratic regression analysis of SGR data to dietary arginine levels reported to be at 18·0 g/kg diet, corresponding to 55·0 g/kg dietary protein for the maximum growth of this fish. Finally, TOR and 4E-BP mRNA levels in different tissues might explain the arginine-enhanced fish growth and digestive and absorptive ability.

Acknowledgements

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