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Effect of tryptophan on growth, intestinal enzyme activities and TOR gene expression in juvenile Jian carp (*Cyprinus carpio* var. Jian): Studies *in vivo* and *in vitro*

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ABSTRACT

This study was conducted both *in vivo* and *in vitro* to investigate the effects of tryptophan on growth performance, digestive and absorptive function and protein synthesis of juvenile Jian carp (*Cyprinus carpio* var. Jian). 1050 juvenile Jian carp (initial weight 7.73 ± 0.03 g) were fed seven isonitrogenous diets with graded concentrations of tryptophan (1.1, 1.7, 2.5, 3.8, 4.9, 6.0, 6.9 g/kg diet) for 8 weeks. Percent weight gain, feed intake and protein retention value were markedly improved, with increases in dietary tryptophan up to 3.8 g/kg diet. Similar trend was found in glutamate–oxaloacetate transaminase (GOT) and glutamate–pyruvate transaminase (GPT) activities, trypsin, lipase and α-amylase activities, Na^+/K^+-ATPase, alkaline phosphatase (AKP), γ-glutamyl transpeptidase and creatine kinase activities, and relative expression of eIF4E-binding protein (4E-BP). On the other hand, feed conversion ratio, plasma ammonia concentration and the relative expression of target of rapamycin (TOR) in different tissue showed an opposite pattern. A series of experiments *in vitro* were then carried out. Compared with the control group, tryptophan supplementation increased 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) OD value, protein content and activity of AKP, GOT, GPT and Na^+/K^+-ATPase in enterocytes and decreased lactate dehydrogenase activity and ammonia concentration in the culture medium. Protein synthesis rate was 17% higher and relative expression of TOR was 28% lower in tryptophan-supplemented than in control carp enterocytes. In conclusion, our results indicate that tryptophan improved fish growth, digestive and absorptive function as well as protein synthesis, which may be partly related to the TOR signaling pathway.

1. Introduction

Tryptophan is an indispensable amino acid in all fish species so far studied (NRC, 2011). Tryptophan deficiency results in depressed growth rate, low feed efficiency and poor protein retention, as reported for the rainbow trout (*Salmo gairdneri*) (Kim et al., 1987) as well as the Indian catfish (*Heteropneustes fossilis*) (Ahmed, 2012). Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are the important transamination enzymes in fish involved in protein and amino acid metabolism (Bystriansky et al., 2007). Teleost fishes excrete their major component of waste nitrogen as ammonia and its production rate being directly related to the rate of amino acid catabolism (Waarde, 1983). Recently, we have shown that optimal level of dietary arginine increased GOT and GPT activities in hepatopancreas and muscle and decreased plasma ammonia levels in Jian carp (*Cyprinus carpio* var. Jian) (Chen et al., 2012). However, little information for tryptophan was available for its influence on fish amino acid metabolism, which warrants investigation.

Fish growth is dependent on digestion ability and absorption function, which were found to correlate with the activity of digestive enzymes and brush border enzymes (Hakim et al., 2006). Digestive enzymes, such as trypsin, chymotrypsin, lipase and amylase, are synthesised in fish exocrine pancreas and secreted into the intestinal lumen (Zambonino-Infante and Cahu, 2001). Others, such as alkaline phosphatase (AKP), Na^+/K^+-ATPase and creatine kinase (CK), are important in the absorptive process in fish (Villanueva et al., 1997). Nevertheless, no reports have been published addressing the effect of dietary tryptophan on fish intestinal enzyme activities. An *in vitro* study showed that tryptophan can activate amylase, lipase, as well as trypsin (Svatos, 1994). In rat pancreas, tryptophan interacted with zymogen granules and caused the release of both trypsinogen

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and chymotrypsinogen (Niederau et al., 1986). Melatonin, an important metabolite of tryptophan, also exerts stimulatory effect on pancreatic enzyme secretion (Jaworek, 2006). Studies on protein structural analysis indicated that tryptophan residues play important roles in catalytic activity, targeting, stabilisation and conformation of several enzymes such as AKP in green crab (*Syclla serrata*) (Zheng et al., 1997), CK in carp (*C. carpio*) (Sun et al., 1998) and ATPase in zebrafish (*Danio rerio*) (Rajarao et al., 2001). These observations suggest that tryptophan may improve the activity of fish digestive enzymes and brush border enzymes. In addition, digestion ability and absorption function were also found to correlate with the growth and development of digestive organs (Pedersen and Sissons, 1984). The development of digestive tract is related to the cell structural integrity in common pandora (*Pagellus eryhtrinus* L.) (Micale et al., 2006). A recent study from our laboratory has shown that structural integrity of fish enterocyte was depressed by oxidative damage (Chen et al., 2009). Melatonin, an indole produced enzymatically from tryptophan, showed the ability of activating antioxidative enzymes and scavenging the radical oxygen species (ROS) to maintain the integrity of rat pancreas (Jaworek, 2006). This provides an example of the possible involvement of dietary tryptophan in growth and development of fish digestive organs, which needs to be investigated.

Fish growth is mainly the result of protein synthesis and deposition (Hochachka and Mommsen, 1995). The regulation of protein synthesis is achieved by alterations in peptide chain initiation through changes in the rate of translation of mRNA (Rhoads et al., 2007). Recent studies indicated that the mammalian target of rapamycin (mTOR) pathway, as an amino acid-sensing mechanism, play a crucial role in this process (Holz et al., 2005; Wullschleger et al., 2006). In mammalian cells, TOR promotes cap-dependent protein synthesis through the phosphorylation and inactivation of its downstream effector 4E-BP (eukaryotic translation initiation factor 4E binding protein) (Wullschleger et al., 2006). Recently, TOR (Genbank accession number: FJ899680) and 4E-BP (Genbank accession number: HQ010440) cDNAs were first cloned from Jian carp in our laboratory. Furthermore, studies from our laboratory have shown that TOR and 4E-BP gene expressions in different tissue were affected by nutrients, such as arginine (Chen et al., 2012) and choline (Wu et al., 2011). Recent studies *in vitro* demonstrated that insulin regulated TOR signaling in rainbow trout (*Oncorhynchus mykiss*) as in mammals (Lansard et al., 2010; Seiliez et al., 2008). Rats fed a tryptophan-deficient diet reduced hepatic protein synthesis, serum insulin, as well as phosphorylation of 4E-BP (Anthony et al., 2001). These observations suggest that tryptophan may regulate fish protein synthesis through the TOR pathway via alterations of TOR and 4E-BP gene expression. To the authors' knowledge, however, there is no information available regarding this.

Therefore, the primary objective of the present study was to investigate the effects of tryptophan on growth performance, digestive and absorptive function and protein synthesis in Jian carp. For that purpose, experiments were performed *in vivo* and *in vitro*, which focused on fish growth, intestinal enzyme activities, TOR and 4E-BP gene expression in different tissues and enterocytes.

2. Materials and methods

2.1. Animals and procedures (in vivo)

The animal protocol was approved by the Animal Care Advisory Committee of Sichuan Agricultural University. Juvenile Jian carp (*C. carpio* var. Jian) were obtained from the Ya'an Hatchery (Sichuan, China). After 4-week adaptation, 1050 carp (mean initial weight 7.73 ± 0.03 g) were randomly allotted into 21 glass aquaria (90 × 30 × 40 cm^3) of seven treatments with three replicates each. Crystalline amino acids (Donboo Amino Acid, Nantong, Jiangsu, China) were supplemented to provide an amino acid profile similar to that of 32% whole chicken egg protein with the exception of tryptophan (Table 1). The casein and gelatin levels were adjusted to provide the lowest level of tryptophan in the basal diet. Tryptophan was increased at the expense of appropriate amounts of alanine, aspartic acid and glutamic acid so as to make all diets isonitrogenous. The experimental diets were designed to provide tryptophan at 1.2, 1.6, 2.6, 3.6, 4.6, 5.6, and 6.6 g/kg of diet. Pyridoxine, inositol, pantothenic acid and riboflavin were supplemented to meet the requirements of Jian carp according to previous studies conducted in our laboratory (He et al., 2009; Jiang et al., 2009; Li et al., 2010; Wen et al., 2009). The procedures for diet preparation and storage were similar to those previously described in other study conducted in our laboratory (Chen et al., 2012). The tryptophan concentrations were determined to be 1.1, 1.7, 2.5, 3.8, 4.9, 6.0 and 6.9 g/kg diet by using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA). All fish were hand-fed with the respective diet six times daily at 07:00, 10:00, 13:00, 16:00, 19:00 and 22:00 h for 8 wk. After feeding 30 min, uneaten feed were removed by siphoning. Water quality was maintained within suitable ranges for Jian carp utilizing a closed-water recirculating system and automatic oxygen supplementation (Jiang et al., 2009). Water temperature, pH and dissolved oxygen were 24 ± 1 °C, 7.0 ± 0.3 and 5.0 ± 0.3 mg/L, respectively.

The procedures of sample collection were similar to those previously described in other study conducted in our laboratory (Jiang et al., 2010). Fish in each aquarium were counted and weighed at the beginning and at the end of the feeding trial after being starved for 12 h. At the beginning of the trial, 30 fish were selected randomly from the same population used in the experiment for the initial body composition analysis. At the end of the trial, 5 fish from each aquarium were collected for the final body composition analysis. Another 15 fish were collected from each aquarium 12 h after the last feeding; then the hepatopancreas, muscle and intestine were removed, quickly frozen in liquid nitrogen and stored at −70 °C. Another 5 fish from each aquarium were collected for obtaining blood samples using heparinised syringes from the caudal

Table 1
Composition of experimental diets.

Ingredients	Diets (g/kg dry diet)						
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Casein	100	100	100	100	100	100	100
Gelatin	100	100	100	100	100	100	100
Amino acid mix[a]	195.87	195.95	197.86	199.77	201.68	203.62	206.22
Corn starch	331.03	330.95	329.04	327.13	325.22	323.28	320.68
α-starch	160	160	160	160	160	160	160
Fish oil	27.9	27.9	27.9	27.9	27.9	27.9	27.9
Soybean oil	18.9	18.9	18.9	18.9	18.9	18.9	18.9
Vitamin premix[b]	10	10	10	10	10	10	10
Mineral premix[c]	10	10	10	10	10	10	10
α-cellulose	20	20	20	20	20	20	20
$Ca(H_2PO_4)_2$	24.5	24.5	24.5	24.5	24.5	24.5	24.5
Ethoxyquin	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Nutrient content[d]							
Calculated crude protein	320.0	320.0	320.0	320.0	320.0	320.0	320.0
Crude protein	317.6	317.6	317.6	317.6	317.6	317.6	317.6
Gross energy (kJ/g)	18.31	18.31	18.33	18.35	18.37	18.39	18.41
Tryptophane	1.1	1.7	2.5	3.8	4.9	6.0	6.9

[a] Amino acid mix (g/kg mixture): lysine 11.733, methionine 7.585, threonine 11.277, arginine 11.367, histidine 5.173, isoleucine 11.945, leucine 18.308, phenylalanine 12.314, valine 13.706, tryptophan variable, cystine 1.207, tyrosine 9.812, alanine variable, aspartic acid variable, glutamic acid variable.

[b] Vitamin mixture (g/kg mixture): retinyl acetate (172 mg/g), 0.800 g; cholecalciferol (12.5 mg/g), 0.480 g; DL-α-tocopherol acetate (50%), 20.000 g; menadione (50%), 0.200 g; thiamin nitrate (98%), 0.063 g; riboflavine (80%), 0.625 g; pyridoxine hydrochloride (98%), 0.755 g; cyanocobalamin (10%), 0.010 g; ascorbyl acetate (92%), 7.247 g; calcium-D-pantothenate (98%), 2.511 g; niacin (98%), 3.176 g; D-biotin (20%), 0.500 g; meso-inositol (98%), 52.857 g; folic acid (96%), 0.521 g.

[c] Mineral mixture (g/kg mixture): $FeSO_4 \cdot 7H_2O$ (19.70% Fe), 76.147 g; $CuSO_4 \cdot 5H_2O$ (25.00% Cu), 1.200 g; $ZnSO_4 \cdot 7H_2O$ (22.50% Zn), 13.334 g; $MnSO_4 \cdot H_2O$ (31.80% Mn), 4.089 g; KI (3.8% I), 2.897 g; $NaSeO_3$ (1.00% Se), 2.500 g; $CaCO_3$ (38.5% Ca), 899.833 g.

[d] Nutrient content: ω3 + ω6, 20; available phosphorus, 6; gross energy was calculated on the basis of fuel values 23.10, 20.21, 24.27, 16.02, 14.81 and 37.65 kJ/g for casein, gelatin, amino acids, α-starch, corn starch and fat, respectively.