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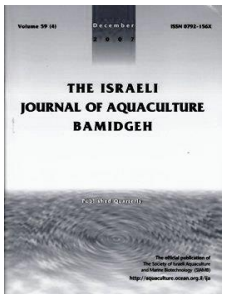

Dietary Vitamin B_6 Requirement of Juvenile Grass Carp (*Ctenopharyngodon idella*)

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Keywords: *Ctenopharyngodon idellua*; vitamin B_6; growth; serum; amino acids.

Abstract

We evaluated the effects of dietary vitamin B_6 levels on growth, survival, and serum biochemical parameters in juvenile grass carp (*Ctenopharyngodon idella*). Fish were fed one of seven purified diets containing 0.12 (control), 1.16, 2.37, 4.82, 9.20, 17.51, or 36.52 mg/kg B_6. We observed abnormal swimming behavior in some fish fed the control diet after 17 days. The survival rate (24.44±1.92%) was low in the control group. Supplementation with vitamin B_6 resulted in significantly higher (P<0.05) weight gain, specific growth rate, final weight, and weight gain rate. Feed efficiency was significantly lower in the fish fed the control diet than the remaining groups (P<0.05). Dietary vitamin B_6 levels had no significant effect on fish body composition or serum albumin and total protein content. Dietary vitamin B_6 supplementation caused a decrease in serum triacylglycerol content and an increase in total cholesterol content, high density lipoprotein cholesterol content, and α-amylase activity levels (P<0.05). Broken-line regression analysis of SGR, HDL-C content, and α-AMY activity, showed that the optimum dietary vitamin B_6 requirement for juvenile grass carp was 1.13, 3.73, 1.57 mg B_6/kg, respectively.

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Introduction

Vitamin B_6 (VB_6), a water-soluble B vitamin also known as pyridoxine, is an organic compound of low molecular weight that is critical for normal metabolism. There are three forms of VB_6: pyridoxine, pyridoxal, and pyridoxamine. In aquatic animals, vitamin B_6 is primarily involved in the metabolism of protein and amino acids, and the synthesis and transformation of unsaturated fatty acids and cholesterol (Giri *et al.* 1997). In fish, deficiencies of vitamin B_6 result in anorexia, slow growth, abnormal swimming behavior, and neurological disorders (Huang *et al.* 2005, Lim et al. 1995, Mohamed 2001).

The dietary vitamin B_6 requirements of aquatic animals have been documented for several species, including *Cyprinus carpio* (Ogino 1965), tilapia (*Oreochromis niloticus* × *O. aureus*) (Shiau & Hsieh 1997), Jian carp (*Cyprinus carpio var. Jian*) (He *et al.* 2009), *Penaeus monodon* (Shiau & Wu 2003), *Heteropneustes fossilis* (Mohamed 2001), *Carassius auratas gibelio* (Wang *et al*. 2011), and *Epinephelus malabaricus* (Wu 2000). The requirement for vitamin B_6 ranges from a few milligrams to tens of milligrams per kilogram.

Grass carp (*Ctenopharyngodon idellus*) is a member of *Osteichthyes, Cypriniformes, Cyprinidae, Ctenopharyngodon*. This species is the most commonly cultured freshwater fish in China. Despite this, its vitamin B_6 requirement has not yet been established. Our objectives were: 1) to determine the effect of dietary vitamin B_6 content on growth, feed utilization, serum components, and body composition of juvenile grass carp and 2) to estimate the dietary vitamin B_6 requirement of juvenile grass carp using growth indices.

Materials and Methods

Diet preparation. The experimental diet formulation is given in Table 1. Casein, gelatin, corn oil and soybean oil were used as sources of dietary protein and lipid, respectively. Dextrin was used as the carbohydrate source. Vitamin B_6 (Hubei Prosperity Galaxy Chemical Co. LTD, China) was supplemented separately to the basal diet at the expense of micro-cellulose to provide concentrations of 0 (control), 1, 2, 4, 8, 16, 32 mg Vitamin B_6 per kg diet, respectively. The actual content of vitamin B_6 in the diets was 0.12, 1.16, 2.37, 4.82, 9.20, 17.51, and 36.52 mg/kg as measured with a high-performance liquid chromatography (SHIMADZU, Japan) (Horwutz 2000). We used microcrystalline cellulose as filler. Concentrations of protein, lipid, and carbohydrate and other nutrients was the same in all diets. The diet was prepared by mixing and pulverizing the ingredients. Distilled water was added to achieve a proper pelleting consistency, and the mixture was further homogenized and extruded through a 2-mm die. The noodle-like diets were dried at room temperature, and then broken into small pieces about 2-3mm size, and stored at -20°C until used.

Table 1. Experimental diet formulation and nutritional ingredients

Ingredient	*Diets (g/kg)*						
Casein	400.0						
Gelatin	60.0						
Dextrin	340.0						
Corn oil and Soybean oil(1:1)	70.0						
Inorganic salts premix	50.0						
vitamin premix	10						
Choline Chloride (37%)	10.0						
Variable ingredient	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ	Ⅶ
Vitamin B6 premix (mg/kg)	0.12	1.16	2.37	4.82	9.20	17.51	36.52
Cellulose	59.88	58.84	57.63	55.18	50.80	42.49	23.48
Nutritional ingredients							
Crude protein	360						
Crude fat	70						
Ash	50						
Moisture	100						

Notes: Vitamin premix formulation: vitamin A 2000 IU/kg; vitamin C 150 mg/kg; vitamin E 100 mg/kg; vitamin K3 5 mg/kg; vitamin D 1000 IU/kg; vitamin B1 10 mg/kg; vitamin B2 20 mg/kg; vitamin B12 0.05 mg/kg; vitamin C 400 mg/kg; Calcium pantothenate 100 mg/kg; Folic acid 5 mg/kg; biotin 1 mg/kg; inositol 500 mg/kg; niacin 150 mg/kg.

Experimental procedure. Juvenile grass carp were supplied by the Yangtze River Fisheries Research Institute, Jingzhou, Hubei Province, China. Initial body weight was about 10 g. The fish were acclimated to the control diet for 14 d, then weighed and randomly assigned into each of the groups in three replicated 400L polyethylene cultivation tanks (21 tanks; 40 fish/tank). Initial body weight of the fish for all was 15.50±0.75 g. Each group was fed one of the experimental diets. All groups were hand fed 3 times per day for 70 d, (8:00–9:00, 12:00–13:00, and 16:00–17:00) at a rate of 1-3% body wet weight per day and weighed every 2 weeks to adjust the feeding level. Water temperature, weight of food consumed, and mortalities were observed and recorded per day.

Sample Collection. Dead fish were removed daily and the survival rate (SR) was calculated. At the end of the feeding trial all fish were fasted for 24 h prior to final sampling and were anesthetized with MS-222 (tricaine methane sulfonate). Then the total number and total body weight of fish from each tank were recorded. At the conclusion of the experiment, 3 fish in each group were randomly selected and blood samples were collected with a pipette or capillary tube by making an incision through the caudal vein. Whole blood was allowed to stand at 4^0C for 4 h then centrifuged at 3000 rpm/min for 10 min to obtain the serum. An additional 3 fish were also sacrificed to measure the growth performance and the whole body composition. These fish were dissected and a sample of muscle tissue was taken from the dorsal region to measure amino acid composition.

Growth performance. The formula of weight gain rate (WGR), feed efficiency (FE), SR, and SGR were as follows:

WGR (%) = 100×(*Wt*- *Wo*) /*Wo*;

FE (%) = 100 ×(*Wt*-*Wo*)/*Wf*;

SR (%) = 100×*Sf/Si*;

SGR (%) = 100×(ln*Wt*-ln*Wo*)/*t*;

t -The number of rearing days (d),

Wt -The total weight of fish in each group after t days (g);

Wo -The initial total weight of fish in each group (g);

Wf -The total weight of feed;

Sf -final number of fish;

Si - initial number of fish.

Analysis of body composition. Crude protein, crude lipid, and ash contents were measured by the Micro-Kjeldahl, Soxhlet, and ignition methods, respectively (Patricia 1998). Moisture was measured by the freeze-drying method, in which samples are freeze-dried for 48 h in a vacuum freeze dryer (Christ Beta 2-4 LD plus LT, Marin Christ Corporation, Osterode, Germany). We measured tissue moisture levels based on the freeze-drying method using a CHRIST type freeze dryer (Christ Beta 2-4 LD plus LT, Marin Christ Corporation, Osterode, Germany). Samples were freeze-dried for 48 h. We measured crude protein levels using the Kjeldahl method, crude fat using the Soxhlet extraction method, and crude ash using the gravimetric method (Patricia 1998).

Serum biochemistry. Serum albumin (ALB), total protein (TP), α-amylase (α-AMY), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and total cholesterol (TC) levels were measured using an automatic biochemical analyzer (Sysmex-800, Sysmex, Japan). The reagents were purchased from Sysmex Corporation.

Analysis of amino acids.

For amino acids (except for tryptophan, glutamine and asparagine), the muscle tissue samples were hydrolyzed with 6N HCl at 110°C for 24 h, then analysis of the amino acids was performed after deriving orthophthaldehyde (OPA, Sigma, St. Louis, USA) using HPLC(SHIMADZU, Japan) following the modified procedure of Gardner and Miller (Gardner and Miller 1980).

Statistical analysis. Data were analyzed by one-way ANOVA and Tukey multiple range tests using SPSS 18.0 for Windows (SPSS Inc, Chicago, Illinois, USA) and are expressed as means with their respective standard deviation (SD). Differences were considered to

be significant at $P<0.05$. Broken-line model (Robbinset al. 2006) was used to estimate dietary vitamin B_6 requirement for juvenile grass carp.

Results

Growth and survival. The fish in groups fed the diets supplemented with vitamin B_6 had significantly higher weight gain and specific growth rate than those in the basal diet group (Table 2; $P<0.05$), however, there was no difference among the groups that were fed with the vitamin B_6 supplemented diets ($P>0.05$). The FE of the basal diet group was significantly lower than the supplemented groups ($P<0.05$). The final weights and WGR were significantly lower in the basal diet group than the other groups ($P<0.05$). By broken-line regression analysis, the relationship between vitamin B_6 content in diets and the specific growth rate is described by the following equations:
$y=0.2906x+2.0042$, $R^2=0.8633$; $y_{max}=2.33$.
The intersected point of these two lines indicated that the lowest dietary vitamin B_6 concentration for maximal specific growth rate was 1.13mg/kg (Fig. 1A).

Table 2. Effects of dietary vitamin B_6 levels on growth and survival of juvenile grass carp. Different superscripts indicate significant differences.

Vitamin B_6 level mg/Kg	Initial weight (g)	Final weight(g)	Weight gain rate (%)	Specific growth rate (%/d)	Feed efficiency (%)	Survival rate (%)
0.12	15.14±0.20	63.12±1.64^b	316.87±11.64^b	2.04±0.04^b	0.73±0.04^a	24.44±1.92^b
1.16	15.53±1.10	79.90±3.18^a	415.72±35.34^a	2.34±0.10^a	0.87±0.03^b	96.67±5.77a
2.37	15.29±0.57	79.39±2.03^a	419.71±23.13^a	2.35±0.06^a	0.88±0.02^b	92.22±8.39a
4.82	15.74±0.82	77.86±2.28^a	411.48±24.23^a	2.33±0.07^a	0.87±0.03^b	97.78±1.92a
9.20	15.34±0.90	77.85±4.66^a	407.36±4.54^a	2.32±0.01^a	0.88±0.04^b	94.44±5.09a
17.51	16.02±0.77	81.06±1.04^a	412.80±25.51^a	2.33±0.07^a	0.85±0.01^b	96.67±4.71a
36.52	15.95±0.91	80.36±3.49^a	403.93±11.09^a	2.31±0.03^a	0.86±0.02^b	95.56±7.69a

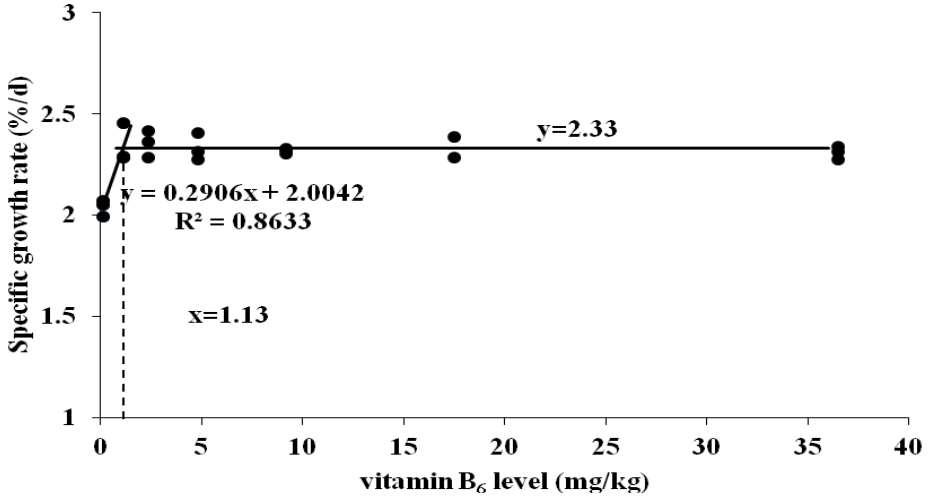

Fig. 1A. Relationship between specific growth rate (%/d) and dietary vitamin B_6 levels.

Vitamin B_6 deficiency. Fish in the control group exhibited abnormal swimming behavior beginning on day 17 and the final survival rates (24.44± 1.92%) were significantly lower than that of the other groups ($P<0.05$).

Serum biochemical parameters. There was no significant difference in serum ALB and TP contents between the treatment and control groups. In contrast, TG levels decreased as dietary vitamin B_6 levels increased and were significantly lower than in the control group. Serum TC content was significantly lower in the control group than in the treatment groups (Table 3; $P<0.05$). HDL-C content and α-AMY activity increased as

dietary vitamin B_6 levels increased (Fig. 1B, 1C). A broken-line regression between vitamin B_6 contents in diets and the HDL-C content or α-AMY activity in serum was used to estimate the optimum vitamin B_6 requirement for juvenile grass carp (Fig. 1B, 1C). The results revealed that the optimum B_6 requirement were 3.73mg/kg for HDL-C and 1.57mg/kg for α-AMY.

Table 3. Effects of dietary vitamin B_6 levels on Serum albumin (ALB), total protein (TP),

Item	*Vitamin B_6 level(mg/Kg)*						
	0.12	*1.16*	*2.37*	*4.82*	*9.2*	*17.51*	*36.52*
ALB(g/L)	15.27±0.57	15.07±0.70	15.07±0.32	15.8±0.56	15.03±0.21	15.4±0.14	15.9±0.30
TP(g/L)	31.53±0.9	32.07±0.42	32.13±0.64	32.27±0.06	31.03±1.12	32.25±0.35	31.15±1.79
TG(mmol/L)	$4.83±0.15^{a}$	$4.46±0.32^{ab}$	$4.4±0.47^{abc}$	$4.41±0.57^{abc}$	$4.05±0.09^{bc}$	$4.17±0.16^{bc}$	$3.76±0.08^{c}$
TC(mmol/L)	$5.81±0.36^{a}$	$6.56±0.35^{bc}$	$6.55±0.21^{bc}$	$6.81±0.26^{bc}$	$6.32±0.13^{b}$	$6.86±0.03^{bc}$	$6.86±0.37^{c}$

triglycerides (TG) and total cholesterol (TC) levels of juvenile grass carp

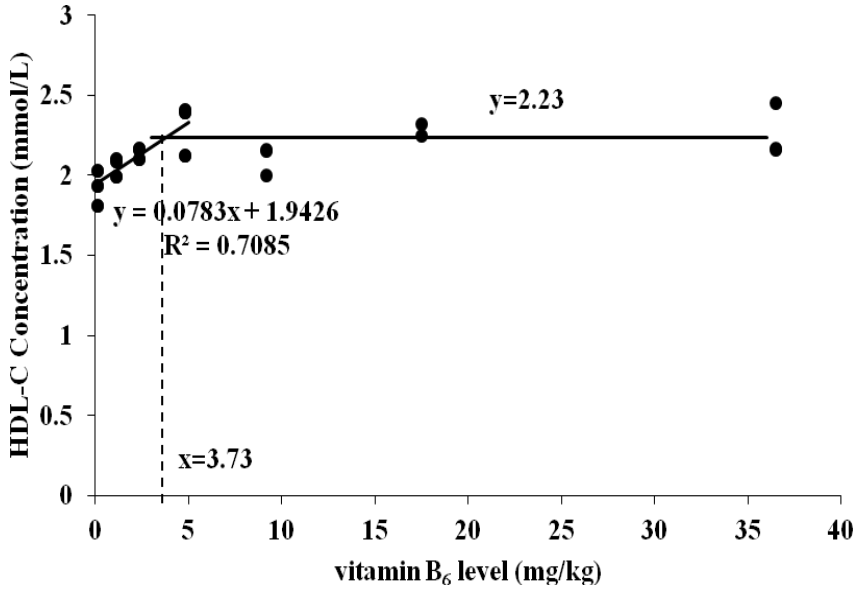

Fig. 1B. Relationship between high-density lipoprotein cholesterol content and dietary vitamin B_6 levels.

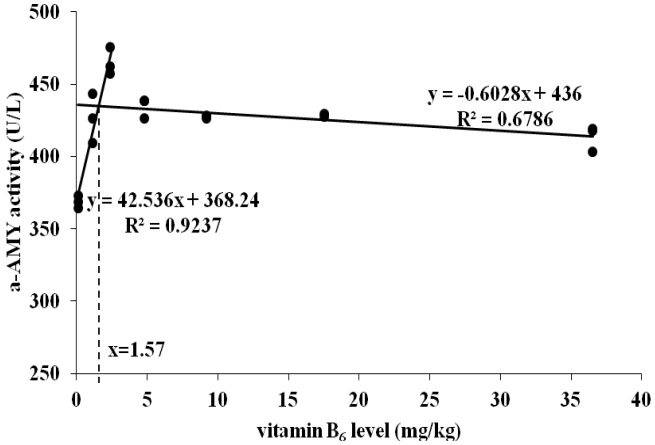

Fig. 1C. Relationship between α-amylase activity and dietary vitamin B_6 levels

Biochemical composition and muscle amino acid composition. Dietary vitamin B_6 supplementation had no effect on whole body crude protein and ash (P>0.05; Table 4). The change of crude fat levels showed a trend from decline to rise as dietary vitamin B_6 levels increased. And crude fat levels were highest in the group supplemented with 9.20 mg VB_6/kg. Moisture in the control group was higher. (Table 4)

Table 4. Effects of dietary vitamin B_6 levels on the body composition of juvenile grass carp

VB_6 level (mg/Kg)	*Moisture (%)*	*Crude protein (%)*	*Crude fat (%)*	*Ash (%)*
0.12	75.61±0.09^{a}	13.74±0.90	6.06±1.05^{a}	3.50±0.07
1.16	73.08±1.33^{bc}	14.34±078	6.29±0.51^{ab}	3.60±0.10
2.37	74.54±0.77^{ab}	14.20±0.90	6.02±0.53^{a}	3.57±0.12
4.82	74.01±1.09^{abc}	14.95±0.43	6.00±0.46^{a}	3.59±0.24
9.20	73.63±1.04^{c}	14.49±0.28	7.30±0.61^{b}	3.48±0.26
17.51	73.63±1.01^{bc}	14.61±0.73	6.25±0.43^{ab}	3.73±0.26
36.52	74.13±0.45^{abc}	14.31±0.02	6.07±0.15^{a}	3.52±0.06

Total muscle amino acid content was significantly higher in the group supplemented with 2.37 mg B_6/kg than the other groups. The addition of vitamin B_6 had significantly effect on muscle ILE, VAL, TYR, SER, PRO, and CYS content. Relative to the control group, SER and PRO content increased significantly (P<0.05) in the treatment groups and ILE, VAL, and TYR were significantly higher in the 9.02, 1.16, 36.52 mg B_6/kg groups (P<0.05). (Table 5).

Table 5. Effects of dietary vitamin B6 levels on muscle amino acid composition of juvenile grass carp

Amino acids	*Vitamin B_6 level (mg/Kg)*						
	0.12	*1.16*	*2.37*	*4.82*	*9.2*	*17.51*	*36.52*
ARG	4.78±0.06^{ab}	4.71±0.13^{ab}	4.93±0.16^{b}	4.74±0.05^{ab}	5.01±0.13^{b}	4.74±0.06^{ab}	4.62±0.49^{a}
LYS	8±0.15^{a}	8.33±0.13^{a}	8.09±0.24^{a}	8.01±0.22^{a}	7.83±0.74^{a}	7.75±0.19^{a}	7.6±0.84^{a}
HIS	2.58±0.03^{a}	2.74±0.06^{a}	2.8±0.05^{a}	2.64±0.17^{a}	2.7±0.14^{a}	2.58±0.12^{a}	2.63±0.17^{a}
ILE	3.5±0.08^{a}	3.5±0.02^{a}	3.59±0.06^{ab}	3.6±0.07^{ab}	4.04±0.31^{b}	3.4±0.04^{a}	3.4±0.27^{a}
LEU	6.66±0.54^{a}	6.44±0.12^{a}	6.63±0.16^{a}	6.43±0.17^{a}	6.35±0.24^{a}	6.36±0.10^{a}	6.21±0.45^{a}
VAL	3.78±0.08^{ab}	4.18±0.19^{c}	3.99±0.10^{bc}	3.89±0.10^{bc}	3.62±0.22^{a}	3.71±0.03^{ab}	3.82±0.14^{abc}
MET	2.16±0.01^{a}	2.14±0.11^{a}	2.31±0.08^{a}	2.18±0.08^{a}	2.26±0.08^{a}	2.23±0.05^{a}	2.17±0.14^{a}
PHE	3.55±0.14^{a}	3.45±0.01^{a}	3.65±0.09^{a}	3.66±0.17^{a}	3.67±0.22^{a}	3.43±0.11^{a}	3.5±0.18^{a}
THR	3.13±0.06^{ab}	3.15±0.07^{ab}	3.28±0.05^{b}	3.16±0.06^{ab}	3.03±0.06^{a}	3.13±0.06^{ab}	3.18±0.18^{ab}
TYR	2.74±0.11^{b}	2.76±0.03^{b}	2.81±0.07^{b}	2.82±0.21^{b}	2.72±0.23^{ab}	2.68±0.11^{b}	2.32±0.42^{a}
DAA							
ASP	7.35±0.12^{a}	7.31±0.17^{a}	7.64±0.19^{a}	7.3±0.11^{a}	7.38±0.16^{a}	7.34±0.12^{a}	7.32±0.52^{a}
SER	3.65±0.86^{a}	4.14±0.12^{bc}	4.36±0.09^{bc}	4.46±0.18^{c}	4.16±0.12^{b}	4.2±0.06^{bc}	4.26±0.22^{bc}
GLU	12.58±0.35^{a}	12.57±0.71^{a}	13.22±0.32^{a}	13.33±0.35^{a}	13.27±0.41^{a}	12.77±0.16^{a}	12.83±0.74^{a}
PRO	2.41±0.04^{a}	2.67±0.24^{b}	3.25±0.08^{cd}	3.02±0.06^{bc}	3.49±0.23^{d}	3.3±0.08^{cd}	3.2±0.30^{cd}
GLY	3.79±0.04^{a}	3.73±0.16^{a}	3.78±0.05^{a}	5.57±0.08^{a}	3.75±0.41^{a}	3.64±0.03^{a}	3.46±0.24^{a}
ALA	4.62±0.10^{a}	4.79±0.10 ^{a}	4.85±0.08^{a}	5.03±0.20^{a}	4.56±0.29^{a}	4.71±0.07^{a}	4.67±0.28^{a}
CYS	0.43±0.05^{ab}	0.75±0.25^{b}	0.37±0.06^{a}	0.36±0.04^{a}	0.5±0.07^{ab}	0.4±0.07^{ab}	0.45±0.01^{ab}
TOTAL	75.68±2.81^{a}	77.34±2.62^{ab}	79.51±1.91^{b}	78.16±2.30^{ab}	78.3±4.06^{ab}	76.34±1.44^{ab}	75.6±5.23^{ab}

Discussion

In our experiment, FE and WGR of the basal diet group were significantly lower. Similarly, in the Indian catfish, Jian carp, Gibel carp, results were similar (He *et al*. 2009; Mohamed 2001; Wang *et al*. 2011). In addition, vitamin B_6 supplementation had a dramatic effect on the survival rate of the experimental fish but had no effect on ash and crude protein content. This is somewhat similar to results in other studies. Pyridoxine deficiency symptoms such as anorexia, poor survival (38.33%) were observed in *Epinephelus coioides* (Huang *et al*. 2005). In tilapia, vitamin B_6 deficiency resulted in

poor survival (43-47%) (Shiau & Hsieh 1997). Vitamin B_6 supplementation had a significant effect on crude protein levels in Jian carp (He *et al*. 2009), and there was a significant effect on crude protein and fat in *Channa argus* (Gui-qin *et al*. 2010). The differences among studies may be a function of differences among species, fish size, and feeding conditions.

Vitamin B_6 plays an important role in fatty acid metabolism. Lipids (including triglycerides, phospholipids, cholesterol, and a small amount of free fatty acids) are primarily transported in the serum as measured in the serum levels of TG, TC, HDL-C.

There was no significant difference in the serum cholesterol among the B_6 supplemented groups. Consistent with other studies, this suggests that vitamin B_6 deficiency has a significant impact on lipid metabolism, although there is a threshold value for vitamin B_6 above which additional supplementation has no effect on TC (Okada & Atsuko 1971; Shijiao & Hong 1992). Cholesterol is transported back to the liver by HDL-C, thereby avoiding the accumulation of harmful cholesterol in the circulatory system and preventing arteriosclerosis (Price & Shah 2001). Analysis of serum triglycerides is an important indicator of health status as increased levels are associated with a higher risk of cardiovascular disease. In our study, HDL-C levels increased as the level of dietary vitamin B_6 increased, whereas TG levels significantly decreased as levels of dietary vitamin B_6 increased. This suggests that vitamin B_6 regulates lipid levels in juvenile grass carp, improves cholesterol transport capacity, and lowers TG levels to reduce fat accumulation and maintain a healthy state.

The α-AMY enzyme plays an important role in maintaining blood glucose levels. In our experiment, the effects of B_6 supplementation on α-AMY activity levels were similar to those in blunt snout bream and *Penaees chinensis* (Zhichang *et al*. 1995). Taken together, these observations suggest that vitamin B_6 promotes metabolic activity by acting as a coenzyme during carbohydrate metabolism.

The muscle amino acid content is influenced by a number of factors, including nutritional composition, temperature, and developmental stage (Coburn 1994, Giri *et al*. 1997). Vitamin B_6 is a coenzyme or cofactor for some amino acid metabolism enzymes such as ornithine decarboxylase, threonine, and serine-degrading enzymes (Okada & Suzuki 1974, Sauberlich 1981). We measured the amino acid levels in juvenile grass carp muscle and noted that the level of glutamate, lysine, and aspartate were higher in B_6 supplemented groups than the control group. This suggests that vitamin B_6 supplementation results in an increase in the content of some amino acids (primarily aliphatic amino acids) in the muscle. Interestingly, an excess of vitamin B_6 had no significant effect on amino acid content. Thus, vitamin B_6 plays a role in regulating amino acid levels in muscle, amino acid metabolism, and meat quality, but the effects are dose dependent.

Feeding a low vitamin B_6 diet did not stop growth in Jian carp (He *et al*. 2009), gibel carp (Wang *et al*. 2011), gilthead bream (Kissil *et al*. 1981), or tilapia (Shiau & Hsieh 1997). We speculate that this is because animals tend to accumulate fat once somatic growth capacity is exceeded. Therefore, calculating the animal's requirement for nutritional factors based on weight gain alone is inappropriate. Instead, there is a need to account for other biological characteristics such as liver function and physiological and biochemical indexes (Phillips Jr & Brockway 1957). In the current study, by broken-line regression analysis of SGR, HDL-C content, and α-AMY activity, it was revealed the optimum dietary vitamin B_6 requirement for juvenile grass carp was 1.13–3.73 mg B_6/kg. This is similar to the estimated requirements for other fish species. For example, the vitamin B_6 requirement is 5 mg B_6/kg in Atlantic salmon (Lall & Weerakoon 1990), 5–6 mg B_6/kg in *chrysophrys major* (Nutrition 1993), 6.07 mg B_6/kg in juvenile Jian carp(He *et al*. 2009), 5–6 mg B_6/kg in *Cyprinus carpio* (Ogino 1965), 3.2 mg B_6/kg in Indian catfish (Mohamed 2001), 7.62–11.36 mg B_6/kg gibel carp (Wang *et al*. 2011), and 1.97 mg B_6/kg in gilthead bream (Kissil *et al*. 1981). The requirement of juvenile grass carp is significantly lower than that of shrimp which require 100 mg B_6/kg (Deshimaru & Kuroki 1979, Wu 2000, Li *et al*. 2010).

To sum up, vitamin B_6 can promote the grass carp growth, regulate the blood glucose and blood triglycerides, and improve muscle amino acids. In this study, the vitamin B_6 requirement of juvenile grass carp is 1.13 - 3.73 mg/kg.

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