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Effect of Dietary Carbohydrate Levels on the Growth and Liver Function of Carp *Cyprinus carpio*

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Keywords*:* corn starch; hepatopancreas histomorphology; enzyme activity; glycogen content; serum biochemistry

Abstract

This experiment was conducted to study the effects of carbohydrate levels on the growth and liver function of Tianjin carp, *Cyprinus carpio* (mean body weight, 60.82 ± 0.38 g). Three diets, including 30% protein and 5.5% fat (dry matter) and 0, 15, or 30% corn starch (carbohydrate source) were provided to control, low carbohydrate, or high carbohydrate treatment groups, respectively. Weight gain, specific growth rate, hepatopancreas somatic index, and liver glycogen content were significantly higher in fish fed the 30% corn starch diet than fish fed 0% corn starch ($P<0.05$) but did not differ between the other two treatment groups. Protein efficiency ratio increased significantly as corn starch level increased ($P<0.05$), but feed conversion ratio showed the opposite trend ($P<0.05$). Glutamic pyruvic transaminase activity in the serum significantly increased with increasing corn starch levels ($P<0.05$), but total protein (decreasing trend) and glutamic-oxaloacetic transaminase (increasing trend) did not differ among dietary treatments. Histomorphological analysis of hepatopancreas sections showed that fish fed 15% or 30% corn starch diets presented mild hydropic degeneration and fatty degeneration or severe fatty degeneration, respectively. In summary, although carbohydrates promote carp growth, they enhance glycogen and fat accumulation in the hepatopancreas, reducing its function.

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Introduction

Carbohydrates play an important role in fish growth (Tan *et al.*, 2007; Cai *et al.*,2009; Asaduzzaman *et al.*, 2009), as they are the main providers of fish body energy (Degani 2006) and important components of body tissues. Carbohydrates are the most economical source of energy. Thus, carbohydrates can substitute fishmeal to a certain extent, reduce feed costs, and reduce the accumulation of total nitrogen and total phosphorus in culture water (Asaduzzaman *et al.*, 2010; Lee& Kim, 2009). However, excessive intake of carbohydrates leads to liver metabolic dysfunction, affects fish growth, survival, and resistance to disease and pathogens (Dixon&Hilton, 1981; Moreira *et al.*, 2008). It is therefore very important to study the effects of dietary carbohydrate levels on the growth and liver function of fish.

Carp (*Cyprinus carpio*) has a long cultivation history in China and the world over. Many unique varieties have been cultivated, and research on their nutrition and feed has become the focus of many studies. The present study focuses on a new variety of carp, the Tianjin carp, which has been bred for 12 years and is currently the fastest growing variety of carp (Liu, 2008). In addition to fast growth, it has a high feed conversion rate, short breeding cycle, low breeding cost, and strong adaptability to the environment. In recent years the study of the effects of dietary carbohydrates on fish focused mainly on blood biochemical indices, gene cloning, and expression of glucose metabolism key enzymes (Nie, 2013; Cai, 2014; Xing, 2015; Zhao, 2009). There are few reports on the effects of dietary carbohydrate levels on liver function and fatty liver disease.

The present study evaluated the effects of dietary carbohydrate levels on growth, liver function, and fatty liver disease of Tianjin carp to find a feed formula that optimizes growth and prevents occurrence of nutritional diseases. The results obtained here provide a theoretical basis for the development and production of carp feed.

Materials and Methods

Experimental animals and feeding management

Three hundred and sixty *C. carpio* individuals were obtained from Tianjin Huanxin Aquatic Breeding Farm (China). They were sterilized with 3-5% salt water and acclimated for a week. During this period fish were fed a basal diet twice daily to satiation. At the beginning of the experiment, fish were fasted for 24 h and then weighed. Thirty fish (mean weight, 60.82 ± 0.38 g) were randomly distributed into each of 12 cages (0.78 × 0.58 × 0.46 m). Each experimental diet was randomly assigned to three of the cages. Fish were fed twice daily (08:00 and 17:00), by hand *ad libitum* (until uneaten feed was visible) for eight weeks, and daily feed intake was recorded. During the experiment, water temperature was 29±1°C, pH was 8.0, dissolved oxygen was above 6.0 mg/L, and ammonia nitrogen was below 0.05 mg/L. Excrement was siphoned out at 10:00 and 1/3 of the water was changed daily.

Experimental diets

Soybean oil was used as the lipid source, and soybean meal, peanut meal, cottonseed meal, rapeseed meal, casein, and fishmeal were used as protein sources.

Corn starch was used as the carbohydrate source. Experimental diets contained three starch levels (0, 15, and 30%) combined with 30% protein and 5.5% lipid. Ingredients and proximate composition varied (Table 1).

Table 1. Formulation and proximate composition of the three experimental diets.

Ingredients	*Diet 1*	*Diet 2*	*Diet 3*
Casein	5.00	5.00	5.00
Fish meal	6.00	6.00	6.00
Peanut meal	35.00	35.00	35.00
Soybean meal	15.00	15.00	15.00
Rapeseed meal	9.00	9.00	9.00
Cotton meal	10.00	10.00	10.00
DDGS	2.00	2.00	2.00
Cellulose	31.80	16.80	1.80
Premix*	1.00	1.00	1.00
Corn starch	0.00	15.00	30.00
Soybean oil	4.00	4.00	4.00
Dicalcium phosphate	2.00	2.00	2.00
Choline	0.20	0.20	0.20
CMC	1.00	1.00	1.00
Total	100.00	100.00	100.00
Proximate composition (%)			
Crude lipid	5.25	5.80	5.41
NFE	14.88	29.69	44.56
Energy (MJ/kg)	11.80	14.56	17.02

*per kg diet: vitamin A, 6000 IU; vitamin B1, 9 mg; vitamin B2, 9 mg; vitamin B6, 7.5 mg; vitamin B12, 0.03 mg; vitamin C 90 mg; vitamin D3, 2000 IU; vitamin E, 60 mg; vitamin K3, 10 mg; D-biotin, 0.15 mg; D-pantothenate, 30 mg; folic acid 3 mg; nicotinamide, 45 mg; inositol, 80 mg; Fe, 140 mg; Cu, 3.5 mg; Mn, 15 mg; Zn, 40 mg; Mg, 100 mg; Co, 0.25 mg; I, 0.5 mg; Se, 0.3 mg; Na, 100 mg; ethoxyquine, 5 mg; choline, 2,000 mg; monobasic calcium phosphate 18,000 mg; and zeolite 19,244 mg.

Determination of growth and condition indices

The weight, body length, and body size of each fish were determined at the beginning and end of the experiment. The hepatopancreas was weighed at the end of the experiment. The number of dead fish and feed intake were recorded during the experiment. The following indices were calculated:

Condition factor (CF,%) = W_t (g)/B^3 (cm) × 100

Hepatosomatic index (HSI,%) = liver weight/W_t × 100

Weight gain percentage (WG,%) = (W_t − W_0)/W_0 × 100

Specific growth rate (SGR,%/d) = 100 × (ln (W_t) − ln (W_0))/t

Feed conversion ratio (FCR,%) = 100 × I_d/(W_t − W_0)

Protein efficiency ratio (PER,%) = (W_t − W_0)/(I_d × P_d) × 100

Survival = N_t/N_0 × 100

where W_t is the mean final weight, W_0 is the mean initial weight, N_t and N_0 are final and initial fish number, respectively, t is the number of experimental days, B is the fork length (cm), I_d is the food intake, and P_d is the protein intake.

Total protein (TP) content and Glutamic-oxaloacetic Transaminase (GOT) and Glutamic Pyruvic transaminase (GPT)activities

At the end of the experiment, nine fish per cage were anesthetized with MS-222. Blood was sampled from the caudal vein and centrifuged at 2500 g for 30 min at 4°C

to obtain blood serum. TP content, GOT, and GTP activities were then determined using commercial kits (Jiang Lai Biotechnology Shanghai Co., Ltd., Shanghai, China).

Hepatopancreas histomorphology

Two fish per cage were randomly selected, anesthetized with MS-222 and their hepatopancreas dissected. Blood was removed from the hepatopancreas using 0.85% saline. This was dried with filter paper before being fixed in Bouin solution and embedded in paraffin. Using a microtome, 5-μm slices were cut and stained using the Hematoxylin and Eosin (HE) method. Slices were then observed and photographed under the optical microscope, connected to a camera. Images were analyzed using software version.

Statistical analysis

All data are expressed as means ± standard deviation and were subjected to analysis of variance (ANOVA) in SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences between means were detected by Duncan's multiple range test and $P<0.05$ was considered significant.

Results

Effects of carbohydrate levels on growth and feed utilization

Growth and feed utilization are shown in Table 2. While WG, SGR, and PER showed an increasing trend, FCR showed a decreasing trend. WG and SGR were significantly higher in fish fed the 30% corn starch diet than in fish fed 0% corn starch ($P<0.05$), but not in fish fed the 15% corn starch diet compared to those fed 0% corn starch ($P>0.05$). Whereas PER increased significantly as corn starch level increased($P<0.05$), FCR showed the opposite trend($P<0.05$). No differences in survival rate (<90%) were observed among treatments($P>0.05$).

Table 2. Effect of the different carbohydrate levels on the growth and feed utilization.

Diet	FBW(g)	WG (%)	SGR (%/d)	FCR (%)	Survival (%)	PER (%)
Diet 1	101.26±5.37^{b}	66.52±0.09^{b}	0.92±0.09^{b}	2.24±0.42^{a}	97.51±0.05	1.46±0.24^{b}
Diet 2	104.32±7.23^{b}	71.77±0.13^{b}	0.97±0.12^{b}	1.66±0.27^{b}	94.26±0.07	2.00±0.26^{a}
Diet 3	119.74±11.77^{a}	96.77±0.19^{a}	1.21±0.18^{a}	1.53±0.22^{b}	94.26±0.07	2.14±0.26^{a}

Note: Data are means ± standard deviation. Within each row, means with different superscripts differ significantly (P <0.05). FBW, Final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio.

Effect of carbohydrate levels on shape index and glycogen

As dietary carbohydrate levels increased, HSI, liver glycogen content, and hepatopancreas fat content increased significantly (P <0.05). There were no significant differences in CF and muscle glycogen content ($P>0.05$) among treatments (Table 3).

Table 3. Effects of the different carbohydrate levels on shape index and glycogen content.

Diet	CF(%)	HSI(%)	HFC(%)	LG(mg/g)	MG(mg/g)
Diet 1	2.42±0.08	1.72±0.37^{b}	4.52±0.39^{c}	1.18±0.26^{b}	0.88±0.20
Diet 2	2.88±0.61	1.79±0.37^{b}	5.66±0.16^{b}	1.71±0.35^{b}	0.83±0.23
Diet 3	2.95±0.56	2.37±0.28^{a}	6.43±0.72^{a}	2.52±0.54^{a}	1.12±0.22

HSI, hepatosomatic index; CF, condition factor; HFC, Hepatopancreas fat content; LG, Liver glycogen; MG, Muscleglycogen.

Effects of carbohydrate levels on serum biochemical indices

Serum GPT activity significantly increased (P<0.05) with increasing corn starch levels and was significantly higher in fish fed the 30% corn starch diet than in fish fed the 0% corn starch diet (P<0.05). Although TP and GOT did not differ among dietary treatments (P>0.05), TP showed a decreasing trend and GOT showed an increasing trend (Table 4).

Table 4. Effect of the different carbohydrate levels on serum biochemical indices.

Diet	TP(g/L)	GPT(U/L)	GOT(U/L)
Diet 1	1.27±0.14	1.14±0.18^{b}	12.76±6.71
Diet 2	1.12±0.14	1.75±0.85^{ab}	12.78±1.23
Diet 3	1.12±0.10	2.87±1.00^{a}	14.80±1.75

TP, Total protein; GPT, Glutamic pyruvic transaminase; GOT, glutamic-oxaloacetic transaminase.

Effect of carbohydrate levels on hepatopancreas histomorphology

Liver cells showed normal morphology in fish fed the 0% corn starch diet (Figure 1a) but in fish fed the 15% corn starch diet, cells showed hydropic and mild fatty degeneration. Karyopyknosis and karyolysis of liver cell nucleus were also observed (indicated by arrows in Figure 1b). Liver cells of fish fed the 30% corn starch diet showed severe fatty degeneration (Figure 1c).

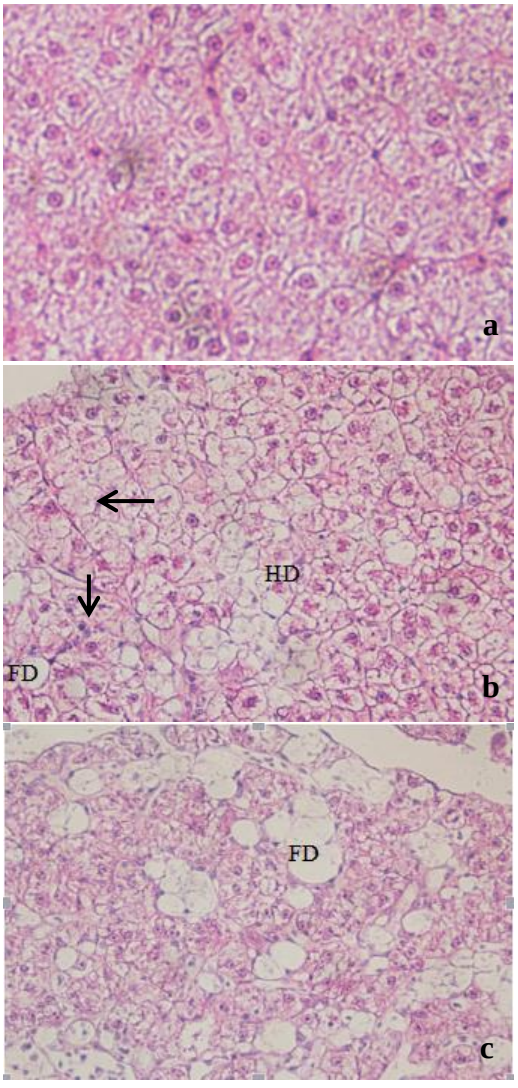

Fig. 1. Microphotographs of the hepatopancreas tissue (HE staining: 400Xmagnification) of carp fed the 0% (a), 15% (b), and 30% (c) corn starch diets.
HD, hydropic degeneration;
FD, fatty degeneration;
downward arrow, karyopyknosis;
left arrow, karyolysis.

Discussion

Effects of carbohydrate levels on growth performance

Carbohydrates are the cheapest source of dietary energy and can play a role in protein reduction, also reducing feed costs, and improving feed protein utilization, if added properly (Asaduzzaman *et al.*, 2013; Cho *et al.*, 2005). The present results showed that WG, SGR, and PER increased significantly with increasing carbohydrate levels, whereas FCR decreased significantly. These results are in agreement with previous findings for juvenile yellow cheek carp, juvenile Darkbarbel catfish, and Grouper (Zhou, 2011; Yang, 2011; Mao *et al.*, 2014). In animals, glycogen is mainly stored in the liver and skeletal muscle and maintained through blood glucose levels. Hepatic glycogen is an emergency energy reserve material that can be rapidly broken down or synthesized in response to stress, thereby regulating the amount of available glycogen (Luo &Xie, 2009). In fish, the liver is also a major place where fat is synthesized from carbohydrates (Lin *et al.*, 1977). Liver glycogen, liver fat, and HSI increased significantly in fish fed the highest corn starch level, indicating that carp can convert excess carbohydrates into glycogen and fat in the hepatopancreas. Similar results were reported for Chinese longsnout catfish (Tan *et al.*, 2007), European seabass juveniles (Moreira *et al.*, 2008; Peres&Oliva Teles, 2002), and Gilthead sea bream (Bou& Todorcevic, 2014; Couto&Enes, 2008). However, it was found that liver glycogen and HSI were not affected by increasing dietary corn starch in African catfish (Ali *et al.* 2004) and in hybrid sriped bass (Nematipour *et al.* 1992). Some studies have also found that HSI and liver glycogen content decreased significantly with increasing dietary carbohydrate levels (Lee & Kim, 2009; Dias & Rueda-Jasso, 2004). These differences might be due to differences in fish species, fish growth stages, and dietary carbohydrates analyzed, to differences in test conditions, and to growth cycles, among other factors. In conclusion, adding carbohydrates to feed might promote carp growth and reduce the amount of protein used, but it might lead to fat accumulation in the hepatopancreas.

Effects of carbohydrate levels on serum biochemistry

Blood biochemistry is closely related to metabolism, nutrition, and diseases. Blood biochemical indices are often used to assess the health and nutritional status of fish (Zhou *et al.*, 2004). Total protein within serum reflects the physiological and metabolic functions of the body (Miu *et al.,* 2011) as proteins are synthesized in the liver. When this is diseased, protein synthesis is low leading to a decrease of TP in the serum. Our results showed that TP was not different among fish the fed different carbohydrate levels, although increasing carbohydrate levels slightly inhibited the synthesis of protein in hepatopancreas thereby reducing serum TP, as reflected in the decreasing trend observed. Similar results have been reported for Allogynogenetic crucian carp (Miu*,* 2009). The most important enzymes in fish liver are GOT and GPT. While GOT exists in the mitochondria and cytoplasm of liver cells, GPT is mainly distributed in the cytoplasm of liver cells. When liver tissues are damaged, GOT and GPT are released into the blood. In the present study, serum GPT activity significantly increased as corn

starch levels increased and GOT activity showed an increasing trend, indicating that excessive carbohydrate levels induce a certain damage to carp hepatopancreas. Some studies also have shown that fish visceral organs are affected by high-carbohydrate diets (Hemre *et al*., 2002).

Effects of carbohydrate levels on hepatopancreas histomorphology

Some studies reported that liver cells showed swelling and fat degeneration, that part of the liver cell membrane ruptured, and cell components moved to the cell edge under dietary carbohydrate levels of 15% and 30% (Cheng *et al*., 2007). Liver lesions are mainly determined by visual inspection of liver and liver sections and by calculating serum indices (Cheng *et al*., 2007; Huang *et al*., 2007; Lin *et al*.,1990). In the present study, liver cells of fish fed the 0% corn starch diet were normal but those of fish fed the 15% corn starch diet showed hydropic and mild fatty degeneration, and karyopyknosis and karyolysis of liver cell nucleus. Liver cells in fish fed the 30% corn starch diet showed more severe fatty degeneration, and both karyopyknosis and karyolysis increased. This is consistent with the significant increase in hepatopancreas fat content and HSI with increasing carbohydrate levels. Therefore, diets with high carbohydrate levels cause some nutritional stress in carp liver tissue and obviously damage it, leading to steatosis.

Conclusions

In fish fed high carbohydrate diets growth performance and feed utilization efficiency improved and glycogen and fat accumulation were induced, while the high carbohydrate diets led to hepatopancreas damage in carp.

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References

Asaduzzaman M., Wahab M.A., Verdegem M.C.J., Benerjee S., Akter T., Hasan M.M. and Azim M.E, 2009. Effects of addition of tilapia Oreochromis niloticus and substrates for periphyton developments on pond ecology andproduction in C/N-controlled freshwater prawn *Macrobrachium rosenbergii* farming systems. *Aquaculture*, 287(3-4):371-380.

Asaduzzaman M., Wahab M.A., Verdegem M.C.J., Adhikary R.K., Rahman S.M.S., Azim M.E. and Verreth J.A.J, 2010. Effects of carbohydrate souurce for maintaining a high C:N ratio and fish driven re-suspension on pond ecology and production in periphyton-based freshwater prawn culture systems. *Aquaculture*, 301(1-4):37-46.

Ali M.Z. and Jauncey K, 2004. Optimal dietary Carbohydrate to lipid ratio in African catfish Clarias gariepinus. *Aquacult Int*, 12(2):169-180.

Bou M., Todorcevic M., Fontanillas R., Capilla E., Gutiérrez J. and Navarro I, 2014. Adipose tissue and liver metabolic responses to different levels of dietary

carbohydrates in gihhead sea bream (*Spares aurata*). *Comp Biochem Physiol A: Mol Integrative Physiol*. 175:72-81.

Cai C.F, 2004. Study on the utilization of dietary carbohydrate by *Mylopharyngodon pieces Richardson* and *Carassius auratus* and their mechanism of metabolism (MD Thesis). East China Normal University, Shanghai, China.

Cai C.F., Chen L.Q., Ye Y.T., Song X.H., Yang C.G. and Chen F.G, 2009. Effects of kind and level of dietary carbohydrate on growth performance and physiological indices of juvenile Black Carp (*MylopharyngodIn Piceus Richardson*). *Chinese J Anim Nutr*, 21(2):212-218.

Cheng C., Xie X.J., Luo Y.P., Mai L.Q. and Lin X.Z, 2007. Effect of dietary carbohydrate level on histology of liver, pancreas and kidney in the Southern Catfish (*Silurus meridiohalis*) Juveniles. *J Southwest Univ (Natural Science Edition)*, 29(6):103-108.

Cho S.H., Lee S.M. and Lee J.H, 2005. Effect of dietary protein and lipid levels on growth and body composition of juvenile turbot (*Scophthalmus maximus* L.) reared under optimum salinity and temperature conditions. *Aquacult Nutr,* 11:235-240.

Couto A., Enes P., Peres H. and Oliva-Teles A, 2008. Effect of water temperature and dietary starch on growth and metabolic utilization of diets in gilthead sea bream (*Spares aurata*) juveniles. *Comp Biochem Physiol, A: Mol Integrative Physiol*, 151:45-50.

Degani G., 2006. Digestible Energy in Dietary Sorghum, Wheat Bran, and Rye in the Common carp *(Cyprinus carpio L.)*. *Isr. J. Aquacult-Bamidgeh* 58(2), 2006, 71-77.

Dias J., Rueda-Jasso R., Panserat S., Conceicao L.E.C., Gomes E.F. and Dinis, M.T, 2004. Effect of dietary carbohydrate-to-lipid ratios on growth, lipid deposition and metabolic hepatic enzymes in juvenile Senegalese sole (*Solea senegalensis, Kaup*). *Aquacult Res,* 35:1122-1130.

Dixon D.G. and Hilton J.W, 1981. Influence of available dietary carbohydrate content on tolerance of waterborne copper by rainbow trout *Salmo gairdneri Richardson*. *J Fish Biol*,19:509-517.

Hemre G.I., Mommsen T.P. and Krogdahl A, 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquacult Nutr*, 8(3):175-194.

Huang K., Yang H.K., Gan H. and Luo Y.J, 2007. Effects of diet-supplemental choline on fatty liver pathological changes in tilapia (*Oreochromis niloticu*s ×*O.aureus*). *J Fish China*, 14(6):257-262.

Lee S.M. and Kim K.D, 2009. Effects of dietary carbohydrate to lipid ratios on growth and body composition of juvenile and grower rockfish, *Sebastes schlegeli*. *Aquacult Res*, 40(16):1830-1837.

Lin D., Mao Y.Q. and Cai F.S, 1990. Nutritional lipid liver disease of grass carp *Ctenopharyngodon idellus*(C. etV.). *Chinese J Oceanol Limnol*, 8(4): 363-374.

Lin H., Romsos D.R. and Tack P.L, 1977. Effects of fasting and feeding various diets on hepatic lipogenic enzyme activities in coho salmon (*Oncorhynchus kisutch* (Walbaum). *J Nutr*, 107(8):1477-1483.

Lin Z., 2008. Tianjin carp became a cultured species. Tianjin Tianjin Science and Technology Yearbook.165.

Luo Y. and Xie X, 2009. Effects of body lipid content on the resting metabolic rate and postprandial metabolic response in the southern catfish *Silurus meridionalis*. *Comp Biochem Physiol A: Mol Integrative Physiol*, 154(4):547-550.

Mao Y.B., Liu H.Y., Tan B.P., Dong X.H., Yang Q.H., Chi S.Y. and Zhang S, 2014. Effects of different dietary carbohydrate levels and starvation on growth and glucose tolerance ability in grouper (*Epinephelus coioides*). *J Fish China*, 38(4): 550-554.

Miu L.H, 2009. Effect of high carbohydrate levels in the dietary on growth performance, immunity and HSP70 of Allogynogenetic crucian carp (MD Thesis). Nanjing Agricultural University, Nanjing, China.

Miu L.H., Liu B., Ge X.P, Xie J., Zhou C.P., Pan L.K., Chen R.L. and Zhou Q.L, 2011. Effect of high carbohydrate levels in the dietary on growth performance immunity and transmission electron microscopy (TEM) on hepatic cell of allogynogenetic crucian carp (*Carassius auratus gibelio*). *J Fish China*, 35(2):221-230.

Moreira I.S., Peres H., Couto A., Enes P. and Oliva-Teles A, 2008. Temperature and dietary carbohydrate level effects on performance and metabolic utilisation of diets in European seabass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, 274(1):153-160.

Nematipour G.R., Brown M.L. and Gatlin D.M, 1992. Effects of dietary carbohydrate: lipid ratio on growth and body composition of hybrid striped bass. *J World Aquacult Soc*, 23(2):128-132.

Nie Q, 2013. Effects of dietary carbohydrates on the activities and gene expression of carbohydrate metabolic enzymes in turbot (*Scophthalmus maximus*) (MD Thesis). Ocean University of China, Qingdao, China.

Peres H. and Oliva-Teles. A, 2002. Utilization of raw and gelatinized starch by European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, 205(3-4):287-299.

Tan Q.S., Xie S.Q., Zhu X.M. and Yang, Y, 2007. Effect of dietary carbohydrate-to-lipid ratios on growth and feed utilization in Chinese longsnout catfish (*Leiocassis longirostris Giinther*). *J Appl Ichthyol,* 23(5):605-610.

Tan X.Y., Luo Z. and Liu Y.J, 2007. Research progress of utilization of carbohydrate in feed. *China Feed*, (6):19-23.

Xing S.J, 2015. Effects of dietary carbohydrates on growth and glycometabolism of Large Yellow Croaker, *Larimichthys croceus* (MD Thesis). Ocean University of China, Qingdao, China.

Yang Y, 2011. Effect of dietary carbohydrate level on growth performance and mRNA expression of several carbohydrate metabolism genes in juvenile Darkbarbel catfish, *Pelteobagrus vachelli* (MD Thesis). East China Normal University, Shanghai, China

Zhao Y.Z, 2009. Advanced in the study of utilization of carbohydrate by black carp (*Mylopharyngodon pieces Richardson*) (MD Thesis). Soochow University, Suzhou, China.

Zhou Y., Guo W.Y., Yang Z.G. and Zhang, K, 2001. Advances in the study of hemotological indices of fish. *J Shanghai Fisheries Univ*, (02):163-165.

Zhou H, 2011. Effect of dietary carbohydrate levels on the growth performance, body compositions and carbohydrate metabolic enzymes activities in juvenile yellow cheek carp (*Elopiclzthys bambusa*) (MD Thesis). Huazhong Agricultural University, Wuhan, China.