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Effects of Oral Glucose Administration on Plasma Biochemical Parameters, Insulin, and Hepatic Metabolic Enzymes Activities in Golden Pompano (*Trachinotus ovatus*)

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Authors' Contribution
CZ designed the experiments, performed the trials and wrote the manuscript. HL, ZH, JW and YW performed the analysis with constructive discussions. WY analyzed the data.

Key words
***Trachinotus ovatus*, Glucose, Tolerance test, biochemical parameters, Hepatic enzyme activities.**

ABSTRACT

Two doses of glucose (335 and 1670 mg of glucose per kg body weight) were orally administered to golden pompano (*Trachinotus ovatus*). Plasma glucose and cholesterol level reached its peak 3h after oral administration with high dose of glucose, respectively ($P<0.05$). Plasma insulin content was significantly lower at 1, 3, 6 and 12 h after oral administration of both doses of glucose ($P<0.05$). Compared to the low dose of glucose group, the high dose of glucose group has lower plasma glucagon content 12 h after oral administration, higher muscle and hepatic glycogen contents 6 h after oral administration ($P<0.05$). The hepatic pyruvate kinase activity peaked 6 h after oral administration in both doses of glucose groups and thereafter started to decrease 24 h after oral administration. While the hepatic PEPCK and G6Pase activities decreased 9 h after oral administration in both doses of glucose groups and then returned to the level at 0 h ($P<0.05$). These results suggested that glucose oral administration resulted in prolonged hyperglycemia and high level of hepatic glycogen in *T. ovatus* which could lead to nutritional stress, and increasing glucose metabolic burden in this species.

INTRODUCTION

In aquaculture, the efficiency of protein utilization for most cultured fish species can be improved by increasing the proportion of energy sources (such as carbohydrate and lipid) in the diet (Cho and Kaushik, 1990; Kaushik and Médale, 1994). As carbohydrates are relatively lower in cost than proteins and lipids, they are thus used in fish diets to improve their physical quality, spare dietary protein and lipid, and provide metabolites for biological syntheses (Wilson, 1994). The utilization of carbohydrate by fish depends on the complexity of the starch (Bergot, 1979; Spannhof and Plantikow, 1983), as well as the gelatinization technology applied to it (Bergot and Breque, 1983). In some species, growth performances were improved in fish fed a diet with an appropriate carbohydrate level compared to a diet without carbohydrates (Hemre *et al*., 2002). In rainbow trout, gelatinized starch has been shown to be as effective as lipid as an energy source (Pieper and Pfeffer, 1980). Fish are generally thought to be not well adapted at the digestive and metabolic levels to deal with high amounts of dietary carbohydrate. At the metabolic level, fish have a limited ability to metabolize glucose. High digestible carbohydrate intake results in a post-prandial hyperglycemia that remains for many hours (Bergot, 1979; Brauge *et al*., 1994; Kaushik and de Oliva Teles, 1985).

Glucose intolerance is a term that refers to the inability of an organism to rapidly deal with a glucose load, which leads to persistent hyperglycemia and in many cases, reduced growth. Glucose intolerance is a clinical term used in the diagnosis of insulin-dependent diabetes mellitus (IDDM) and is evaluated by the use of a glucose tolerance test (GTT) (Moon, 2001; Shahida *et al.*, 2017). Glucose tolerance tests have been carried out in several fish species to study the metabolic utilization of glucose (Furuichi and Yone, 1981; Garcia-Riera and Hemre, 1996;

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Harmon *et al*., 1991). Although, in each case, glucose administration resulted in prolonged hyperglycemia, there were still marked differences among species.

Golden pompano (*Trachinotus ovatus*) belongs to the family Carangidae (Sun *et al*., 2014; Tutman *et al*., 2004). *T. ovatus* is a carnivorous fish that preys mainly on zooplankton and small crustaceans, shellfish, and fish (Liu and Chen, 2009; Su *et al*., 2012; Zheng *et al*., 2014). Zhou *et al*. (2015) have reported that the optimum carbohydrate requirement of golden pompano ranged from 11.2% to 16.8%. The aim of this study was to determine glucose tolerance in *T. ovatus* in order to gain further knowledge on glucose regulation in marine fish species, as well as interactions among plasma glucose, insulin, glucagon, triglyceride, cholesterol, hepatic and muscle glycogen, and four kinds of key hepatic enzymes related to glucose metabolism.

MATERIALS AND METHODS

Experimental animals and procedures

The animals used in this experiment were golden pompano (*Trachinotus ovatus*). The experiment was carried out at the Shenzhen Experimental Station of South China Sea Fisheries Research Institute of CAFS, in a water recirculation system equipped with 14 cylindrical fiberglass tanks of 300 L capacity. Fish were stocked in fourteen tanks each with 20 *T. ovatus*. Before the trial, the fish (average weight, 33.47 ± 0.63g) were allowed to adapt to the experimental facilities for two weeks, and during this period were fed by hand, twice a day, to near satiety with an experimental diet. This diet had a protein content of 42% and a lipid content of 6%, and was presented as dry pellets, manufactured in the laboratory using a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China). During the trial, water temperature averaged 27.3 ± 0.5 °C and salinity 28 ± 1‰.

After the acclimation period, fish were fasted for 2 weeks, lightly anaesthetized with diluted eugenol (1:10,000; Shanghai Reagent Corp., China), immediately weighed and orally administrated with two dose glucose (335 and 1670 mg of glucose per kg body weight, respectively). Fish were then placed back in the experimental tanks. Fish experiments were performed in accordance with the guidelines for fish research from the animal ethic committees at South China Sea Fisheries Research Institute.

Sample collection

Blood, muscle and liver samples were collected just before time 0 and 1, 3, 6, 9, 12 and 24 h after glucose oral administration. At each sampling time, four fish were sampled and individually weighed. Blood was collected from the caudal vein using heparinized syringes, immediately centrifuged and the plasma frozen for analysis. The liver was excised, frozen in liquid nitrogen, and stored at -20°C until analysis. Muscle samples were taken from the lateral dorsal part of the body behind the dorsal fin, and stored at -20°C for analysis.

Plasma biochemical parameter analysis

Plasma glucose, cholesterol and triglyceride contents were measured using the glucose oxidase method (Lott and Turner, 1975), the enzymatic (cholesterol oxidase) and colorimetric method (Patsch *et al*., 1976), and the enzymatic (glycerol phosphate oxidase) and colorimetric (PAP) method (Nägele *et al*., 1983), respectively, using test kits purchased from Junshi Biotechnology Co., Ltd. (Shanghai, China).

Hepatic and muscle glycogen analysis

The hepatic and muscle glycogen contents were determined spectrophotometrically at 620 nm using the anthrone reaction method as previously described in previous study (Plummer, 1987).

Plasma hormones assays

Plasma insulin was estimated by radioimmunoassay (RIA) using a test kit (Beijing Beifang Biotech Research Institute, Beijing, China) and following the method described by Clark and Hales (1994). The plasma glucagon level was measured by the double antibody sandwich method using Glucagon ELISA detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Optical densities were both measured at 450 nm.

Hepatic enzyme activities analysis

A frozen sample of liver was homogenized (dilution 1/10) in ice-cold buffer consisting of 80 mM Tris (pH 7.5), 5 mM EDTA, 1mM KH_2PO_4, 2mM $NaHCO_3$ and 1.4 mM dithiothreitol. The homogenate was separated and divided into two parts. One part of the homogenate was centrifuged at 4,000 rpm for 10 min at 4°C (Moreira, *et al*., 2008), and then the supernatant was centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was separated and divided into two parts for measurement of hexokinase (HK) and pyruvate kinase (PK) activities. The activities of hepatic HK (EC 2.7.1.1) and PK (EC 2.7.1.40) were determined using kits purchased from Nanjing Jiancheng Bioengineering Institute following the procedures described in previous studies by Foster and Moon (1986) and Tranulis *et al*. (1996), respectively. The enzyme activities were expressed per mg of total protein (specific

activity). The total protein content in crude extracts was determined at 30°C using bovine serum albumin as a standard based on the previous method (Bradford, 1976).

The other part of homogenate was centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was separated and divided into two parts for measurement of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) activities. The activities of PEPCK (EC4.1.1.32) and G6Pase (EC3.1.3.9) were measured by the double antibody sandwich method using PEPCK and G6Pase ELISA detection kits (Jijin Chemical Technology Co., Ltd., Shanghai, China), respectively. Optical densities were both measured at 450 nm.

Statistical analysis

The SPSS (version 19.0, Chicago, IL, USA) software was used to perform Duncan's multiple range tests and Independent-Samples t-tests to determine differences among treatments. Diverse little letters show significant differences (P<0.05) in different glucose dose groups of each sampling point in Duncan's multiple range tests. Significant differences (P<0.05) between values obtained from control and high glucose dose groups are marked by asterisks above histogram bars in Independent-Samples t-tests. All the results were expressed as means ± standard error ($\bar{X}$±SE).

RESULTS

Plasma glucose, cholesterol and triglyceride levels

Plasma glucose content at 1 and 3 h after oral administration was significantly higher than that at 0 h in the high dose of glucose group and then plasma glucose content in the high dose of glucose group returned to the level at 0 h (P<0.05) (Fig. 1A). Similarly, plasma glucose content at 1 and 3 h after oral administration was significantly higher than that at 0 h in the low dose of glucose group and then plasma glucose content in the low dose of glucose group returned to the level at 0 h (P<0.05) (Fig. 1A). Plasma glucose was significantly higher in the high dose of glucose group than that in the low dose of glucose group at 6 h (P<0.05) (Fig. 1A). Plasma cholesterol content in the high dose of glucose group at 1, 3, 12 and 24 h after oral administration was significantly higher than that at 0 h in the high dose of glucose group (P<0.05) (Fig. 1B). There was no significant change in plasma cholesterol content in the low dose of glucose group (Fig. 1B). Plasma triglyceride content at 1, 3, 6, 9, 12 and 24 h after oral administration was significantly higher than that at 0 h in the high dose of glucose group (P<0.05) (Fig. 1C). Plasma triglyceride content at 6 h after oral administration was significantly higher than that at 0 h in the low dose of glucose group (P<0.05) (Fig. 1C). Plasma triglyceride content was significantly higher in the high dose of glucose group than that in the low dose of glucose group at 1 h (P<0.05) (Fig. 1C).

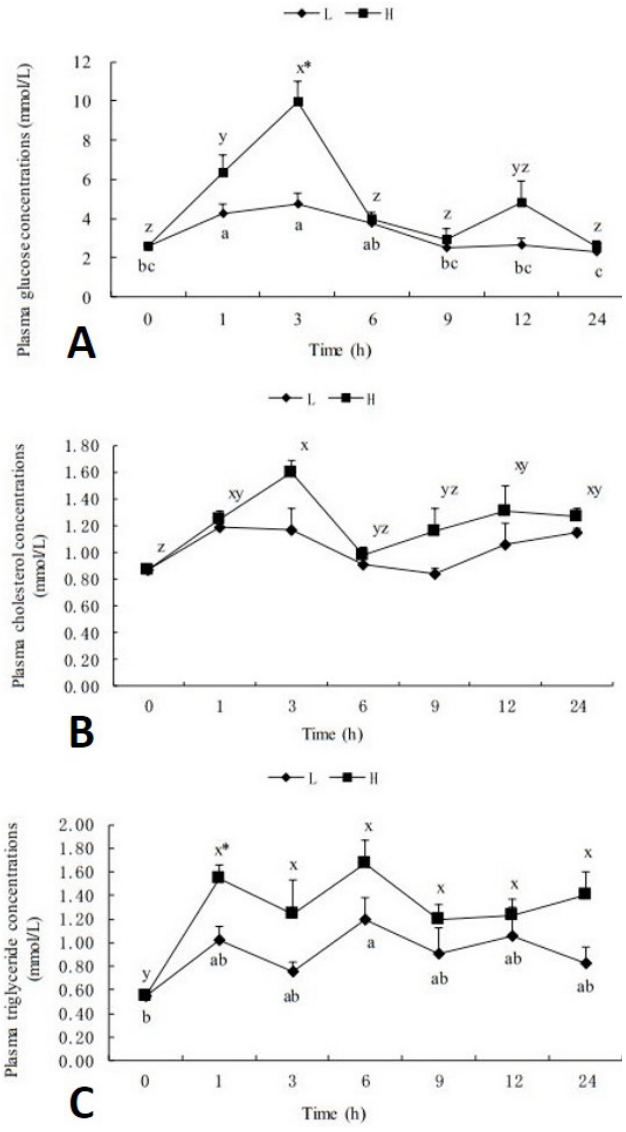

Fig. 1. The change of plasma glucose, cholesterol and triglyceride in golden pompano *Trachinotus ovatus* over time after oral administration of two doses of glucose. Values with different superscripts in each group indicate significant difference (P < 0.05).

Plasma insulin and glucagon levels

Plasma insulin content at 1, 3, 6 and 12 h after oral administration was significantly lower than that at 0 h in the high dose of glucose group and then plasma insulin content in the high dose of glucose group returned to the level at 0 h

(P<0.05) (Fig. 2A). Plasma insulin content at 1, 3, 6, 9, 12 and 24 h after oral administration was significantly lower than that at 0 h in the low dose of glucose group (P<0.05) (Fig. 2A). Plasma glucagon content at 1, 9, 12 and 24 h after oral administration was significantly higher than that at 6 h in the low dose of glucose group and then plasma glucagon content in the low dose of glucose group returned to the level at 0 h (P<0.05) (Fig. 2B). Plasma glucagon content at 6 h after oral administration was significantly lower than that at 0 h in the high dose of glucose group and then plasma glucagon content in the high dose of glucose group returned to the level at 0 h (P<0.05) (Fig. 2B). Plasma glucagon content was significantly lower in the high dose of glucose group than that in the low dose of glucose group at 12 h after oral administration (P<0.05) (Fig. 2B).

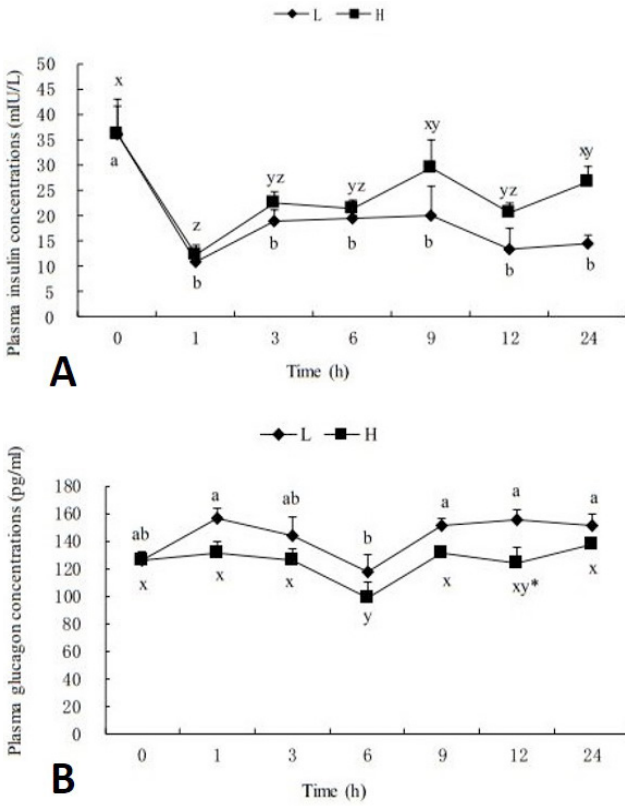

Fig. 2. The change of plasma insulin and glucagon in *T. ovatus* over time after oral administration of two doses of glucose.

Hepatic and muscle glycogen contents

The hepatic glycogen content at 6 h after oral administration was significantly higher than that at 0 h in the high dose of glucose group and then hepatic glycogen content in the high dose of glucose group returned to the level at 0 h (P<0.05) (Fig. 3A). The hepatic glycogen content at 3, 9 and 12 h after oral administration was significantly lower than that at 0 h in the low dose of glucose group (P<0.05) (Fig. 3A). The hepatic glycogen contents were significantly higher in the high dose of glucose group than those in the low dose of glucose group at 3 and 6 h after oral administration, respectively (P<0.05) (Fig. 3A). There was no significant change in muscle glycogen contents in both groups (P>0.05) (Fig. 3B). The muscle glycogen contents were significantly higher in the high dose of glucose group than those in the low dose of glucose group at 1 and 6 h after oral administration, respectively (P<0.05) (Fig. 3B).

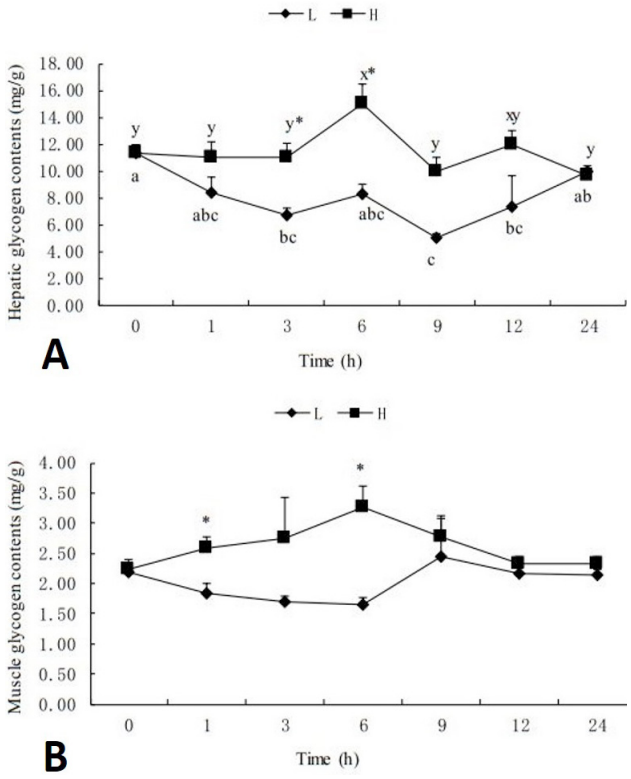

Fig. 3. The change of hepatic and muscle glycogen in *T. ovatus* over time after oral administration of two doses of glucose.

Hepatic HK, PK, PEPCK and G6Pase activities

There was no significant change in hepatic hexokinase activities in both groups (P>0.05) (Fig. 4A). The hepatic hexokinase activity was significantly higher in the high dose of glucose group than that in the low dose of glucose group at 6 h (P<0.05) (Fig. 4A). The hepatic pyruvate kinase activity in the high dose of glucose group at 6, 9, 12 and 24 h after oral administration was significantly higher than that at 0 h in the high dose of glucose group (P<0.05) (Fig. 4B). The hepatic pyruvate kinase activity in the low dose of glucose group at 6 h after

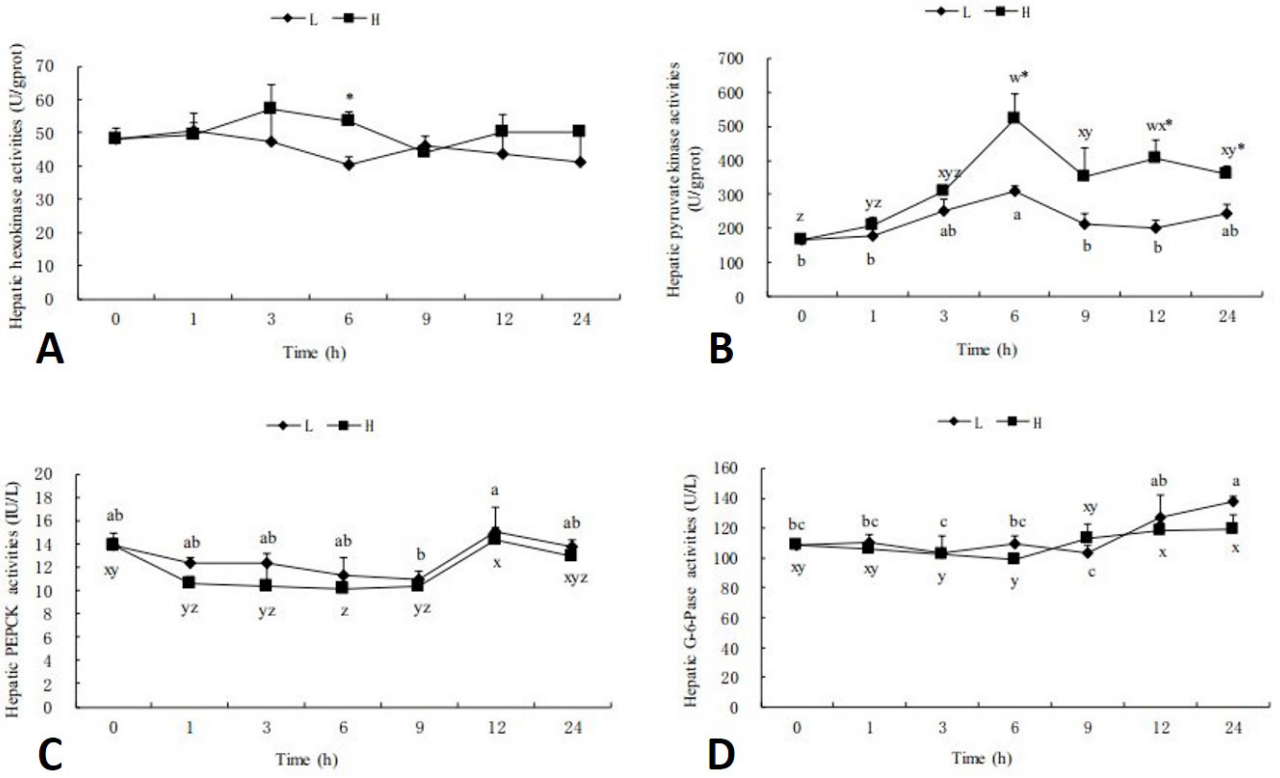

Fig. 4. The change of hepatic hexokinase (HK), pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) activities in *T. ovatus* over time after oral administration of two doses of glucose.

oral administration was significantly higher than that at 0 h in the low dose of glucose group (P<0.05) (Fig. 4B). The hepatic pyruvate kinase activities were significantly higher in the high dose of glucose group than that in the low dose of glucose group at 6, 12 and 24 h, respectively (P<0.05) (Fig. 4B). The hepatic PEPCK activity at 0 and 12 h after oral administration was significantly higher than that at 6 h in the high dose of glucose group and then hepatic PEPCK activity in the high dose of glucose group returned to the level at 0 h (P<0.05) (Fig. 4C). The hepatic PEPCK activity at 9 h after oral administration was significantly lower than that at 0 h in the low dose of glucose group and then hepatic PEPCK activity in the low dose of glucose group returned to the level at 0 h (P<0.05) (Fig. 4C). The hepatic G6Pase activities at 12 and 24 h after oral administration was significantly higher than those at 3 and 6 h in the high dose of glucose group, respectively (P<0.05) (Fig. 4D). The hepatic G6Pase activity at 24 h after oral administration was significantly higher than that at 0, 1, 3, 6 and 9 h in the low dose of glucose group, respectively (P<0.05) (Fig. 4D).

DISCUSSION

The results in present study showed that the changes of plasma glucose levels in both glucose doses groups had similar trend, namely the rapid increase during 3h to the maximum peak, and then returned to the level at 0 h. This agreed well with the results of glucose tolerance in other fish species (Cai and Wang, 1998; Cai *et al*., 2002; Furuichi and Yone, 1981; Huang *et al*., 2005; Peres *et al*., 1999; Yang *et al*., 2012). However, the recovery time of plasma glucose level varied in different fish species and different methods of administration in glucose tolerance test. For the method of oral administration, the recovery time to normal level was 5 h in carp (*Cyprinus carpio*) (Furuichi and Yone, 1981), about 6 h in grouper (*Epinephelus coioides*) (Yang *et al*., 2012), about 10 h in grass carp (*Ctenopharyngodon idella*) (Huang *et al*., 2005), nearly 10 h in black carp (*Mylopharyngodon piceus*) at low dose of glucose (420 mg/kg body weight), more than 10 h in black carp (*Mylopharyngodon piceus*) at high dose of glucose (1370 mg/kg body weight) (Huang *et al*., 2005). For the method of intraperitoneal injection administration (1000 mg/kg body weight), plasma glucose levels of turbot

(*Scophthalmus maximus*) returned to pre-injection levels took about 24h (Garcia-Riera and Hemre, 1996), gilthead bream and European seabass 12 h (Peres *et al.*, 1999). The previous study showed that plasma glucose levels of rainbow trout (*Oncorhynchus mykiss*), intraperitoneal injected of 3000 mg/kg body weight of glucose, returned to pre-injection level required 18h (Harmon *et al.*, 1991). The difference of response time to restore glucose levels after glucose load could result from different fish species and the method used to increase glucose content. In this study, plasma cholesterol content in the high dose of glucose group after oral administration was significantly increased. There was no significant change in plasma cholesterol content in the low dose of glucose group. Similar result was observed in previous study on tilapia (*Oreochromis miloticus*) (Liu *et al*., 2012). Vertebrates usually had the ability converting excess glucose into lipid, but in fish, this ability varied in different fish species (Kumar *et al*., 2010). The results in our study showed that the plasma triglyceride contents of *T. ovatus* in both doses of glucose were increased 0-9 h after oral administration. The plasma triglyceride content was significantly higher in the high dose of glucose group than that in the low dose of glucose group at 1 h. However, the results in previous study on grouper (*Epinephelus coioides*) showed that the plasma triglyceride contents in both doses of glucose were increased 0-15h after oral administration, especially the low dose glucose group obvious (Yang *et al*., 2012). The results in previous study on grass carp (*Ctenopharyngodon idella*) showed that the plasma triglyceride contents in both doses of glucose (1370 mg/kg and 420 mg/kg) were significantly decreased 0-15 h after oral administration (Huang *et al*., 2005). The plasma triglyceride contents of black carp in high dose of glucose (1370 mg/kg) were increased 0-15h after oral administration, while decreased in low dose of glucose (420 mg/kg) (Huang *et al*., 2005). The plasma triglyceride content of gibel carp was increased within 0-1 h after oral administration, and then decreased within 1-4 h (Cai *et al*., 2002). Harmon and Sheridan (1992) observed that glucose stimulates fatty acid reesterification and directly enhances net lipolysis in trout liver incubated *in vitro*. The reason of these inconsistent results need further study.

The plasma glucose levels in human beings are regulated by many hormones, such as glucagon, glucagon-like peptide (GLP), insulin-like growth factor (IGF), growth hormone, the somatostatins, and so on (Moon, 2001). In mammals, insulin can promote glucose uptake and utilization of tissues and cells, promote glucose synthesis of glycogen stored in the liver and muscles, while suppress gluconeogenesis, promote glucose into fatty acids stored in adipose tissue, thereby lowering plasma glucose level. Contrary, glucagon has the opposite effect on regulating plasma glucose level (Yang *et al*., 2012). Thus, insulin in mammals is considered to be one of most important hormones to maintain plasma glucose level, whereas it is different in the fish. Some studies indicated that the fish had low insulin levels (Wilson and Poe, 1987), fewer number of insulin receptors with weak affinity (Gutiérrez *et al*., 1991; Mommsen and Plisetskaya, 1991). However, Thorpe and Ince (1976) observed that the plasma insulin levels in Pacific cod (*Gadous macrocephaius*) and the European eel (*Anguilla anguilla*) were higher than that of human being. In contrast, in the present study, the plasma insulin levels in *T. ovatus* was lower than that of human being, which was similar to previous studies on grouper (*Epinephelus coioides*) and pike (*Esox lucius*) (Thorpe and Ince, 1976; Yang *et al*., 2012). These opposite results can be related with species differences. In the present study, plasma insulin levels of *T. ovatus* decreased significantly with increasing plasma glucose levels within 0-1 h after oral administration, which is in agreement with previous study on Chinook Salmon (*Oncorhynchus tshawytscha*) after oral administration (Mazur *et al*., 1992). The results observed in this study might be related with sampling time and/or season. Previous studies showed that daily and annual variations of plasma insulin levels in European seabass, although in this species insulin levels peaked during the dark period (Gutiérrez *et al*., 1984, 1987). Another explanation is that decrease in plasma insulin level may be related with somatostatin secretion. Ronner and Scarpa (1984) observed that lots of somatostatin was released after the isolated splenic Brockmann body of channel catfish (*Ictalurus punctatus*) was perfused *in vitro*. Sheridan *et al*. (1991) indicated that lots of somatostatin secretion in fish inhibited secretion of insulin, leading to the insulin at a low level. In addition, some studies showed that there are two explanations about relationships between the change of insulin and plasma glucose as follows: This delayed insulin secretion in teleost fish was more slowly than mammals (Enes *et al*., 2012; Furuichi and Yone, 1981). The plasma insulin levels remained constant for a long time in fish after glucose load (Enes *et al*., 2011). The insulin seemed not to be directly involved in clearance of glycemic load (Mazur *et al*., 1992). Legate *et al*. (2001) considered that it is not the main factor of glucose intolerance in fish.

Glycogen is the storage form of carbohydrate in the body, mainly in liver glycogen, muscle glycogen form. The hepatic glycogen synthesis and degradation is mainly to maintain a relatively constant plasma glucose concentration. The synthesis and degradation of muscle glycogen is primarily to provide ATP for the muscle. Many studies found that the postprandial plasma hepatic glycogen level of the fish was increased after feeding dietary carbohydrates (Hemre *et al*., 2002; Peres *et al*., 1999; Wilson, 1994). The persistent high level of

plasma glucose and liver glycogen are adverse to normal metabolism. In the present study, the liver glycogen of *T. ovatus* peaked 3 h after oral administration, and then decreased significantly. Glucose injection had no effect on muscle glycogen levels. These results agree with the observations of early studies, where the muscle glycogen level was not increased by high-carbohydrate diet (Banos *et al*., 1998). However, Cai *et al*. (2002) observed that high dose of glucose administrated had decreased the hepatic glycogen level, whereas the low dose of glucose administrated had no significant effect. Similar results were found in glucose tolerance study on gilthead sea bream (*Sparus aurata* L.) (Peres *et al*., 1999). Carbohydrate is one of important energy sources in fish, after the intake of carbohydrates to meet the energy requirement of fish, the excess carbohydrates were transported to liver and muscle stored as glycogen. However, the process of the conversion is not very rapid, 3 h after oral administration plasma glucose level started to increase to a peak value, whereas liver glycogen content started to increase to a peak value 6 h after oral administration, which suggested that *T. ovatus* had limited ability to metabolize glucose.

Pyruvate Kinase (PK) is one of important rate-limiting enzymes involved in glycolysis (Yuan *et al*., 2013). Increased PK showed enhancement of glycolysis. Dietary carbohydrate had some impact on hepatic PK activity in fish. Early study showed that compared with no carbohydrate group, the hepatic pyruvate kinase activity of topmouth culter (*Erythroculter ilishaeformis* Bleeker) was increased significantly in high carbohydrate group (Ge *et al*., 2007). Cai (2004) observed that the hepatic and muscle PK activities in black carp (*Mylopharyngodon pieces*) increased significantly with increasing dietary carbohydrate levels. Liu *et al*. (2012) indicated the hepatic PK activity in tilapia was increased to a peak 6 h after glucose injection. In this study, the hepatic PK activity in *T. ovatus* was increased to a peak 6 h after glucose administration. These results suggested that high concentration of glucose may increase glycolytic capacity and increase the utilization of carbohydrate, but the enhancement of enzyme activity still lags behind the absorption of carbohydrate in fish, which was adverse to carbohydrate metabolism.

PEPCK is key enzyme in the lyase family used in the metabolic pathway of gluconeogenesis. It converts oxaloacetate into phosphoenolpyruvate and carbon dioxide (Méndez-Lucas *et al*., 2013, 2014). PEPCK is most abundant in the liver, kidney, and adipose tissue (Chakravarty *et al*., 2005). Previous showed that the hepatic PEPCK activity in mammals decreased significantly after feeding high-carbohydrate diet, increased after fasting or feeding high-protein and low-carbohydrate diet (Hanson and Reshef, 1997). Li *et al*. (2015) reported that the hepatic PEPCK activity all decreased with increasing dietary carbohydrate levels in three fish species. Ge *et al*. (2007) observed that the hepatic PEPCK activity of topmouth culter (*Erythroculter ilishaeformis* Bleeker) showed a downward trend with increasing dietary carbohydrate levels. In agreement with these observations the present study in *T. ovatus* showed that the hepatic PEPCK activity showed a downward trend within 0-9 h after glucose administration, and then an upward trend 12 h after glucose administration, which suggested that the gluconeogenesis was inhibited firstly and then gradually increased.

G6Pase is an enzyme in gluconeogenesis and glycogenolysis, which plays a key role in the homeostatic regulation of blood glucose levels (Nordlie and Sukalski, 1985). It was reported that dietary nutrients affect G6Pase expression and/or activity in mammals, but complicated in fish. In the present study, we observed that the hepatic G6Pase activity showed a downward trend within 0-9 h after glucose administration, and then an upward trend within 0-9 h after glucose administration. Similarly, Panserat *et al*. (2000) reported that the hepatic G6Pae activity and/or expression of rainbow trout fed 8-20% dietary carbohydrate for 10-week showed no significant difference. In gilthead bream, Metón *et al*. (2004) reported that the dietary carbohydrate had no effect on the expression of hepatic G6Pase. However, other researchers observed that the hepatic G6pase activity was inhibited significantly in carp fed diets contained starch, glucose and fructose (Furuichi and Yone, 1982; Shikata *et al*., 1994).

CONCLUSION

From the results of this trial, it can be concluded that the prolonged hyperglycemia in *T. ovatus* resulted from high dose of glucose administration. The increasing plasma insulin level and hepatic PK activity were relatively delayed after glucose administration, which limited the glycolysis of glucose. The prolonged hyperglycemia and high level of hepatic glycogen led to nutritional stress, and increasing glucose metabolic burden in this species. Further studies concerned the molecular mechanism of glucose regulation in marine fish species should be conducted to elucidate this.

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Statement of conflict of interest

The authors declare no conflict of interest for this research work.

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