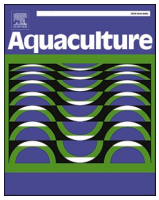
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Modulation of growth, antioxidant status, hepatopancreas morphology, and carbohydrate metabolism mediated by alpha-lipoic acid in juvenile freshwater prawns *Macrobrachium nipponense* under two dietary carbohydrate levels

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ABSTRACT

Carbohydrate is commonly used in aquatic organism as the cheapest energy source. However, most aquatic animals are low efficient in utilizing dietary carbohydrate for energy provision. Therefore, improving the utilization efficiency of high carbohydrate by aquatic organisms is important for sustainable aquaculture development. Alpha-lipoic acid (α-LA) is a cofactor of key enzymes involved in glucose metabolism. However, its role in regulation of carbohydrate metabolism in aquatic animals is currently unknown. This study investigated the effects of α-LA on growth, antioxidant status, hepatopancreas morphology, and carbohydrate metabolism of freshwater prawns *Macrobrachium nipponense* under two dietary carbohydrate levels. Six diets were formulated using casein and fish meal as the protein sources, fish oil and soybean oil as the lipid sources, and corn starch as the carbohydrate source, at two carbohydrate levels (15% and 30%) and three concentrations of α-LA supplementation (0, 700, and 1400 mg/kg). Each experimental diet was fed to juvenile prawns (mean weight 0.050 ± 0.003 g), twice daily to apparent satiation, in five replicates. In the 15% (low)-carbohydrate groups, weight gain was significantly reduced in prawns fed 1400 mg/kg α-LA compared with 0 or 700 mg/kg α-LA, but there was no significant effect in the 30% (high)-carbohydrate groups. Survival was significantly higher among prawns fed 700 mg/kg α-LA compared with 0 or 1400 mg/kg α-LA regardless of the carbohydrate level. The levels of dietary carbohydrate and α-LA also significantly affected the activities of key enzymes involved in glycolysis and the citric acid cycle. The activities of hexokinase and pyruvate kinase activities were highest in prawns fed l400 mg/kg α-LA, in both the low- and high-carbohydrate groups. Supplementation with 700 or 1400 mg/kg α-LA significantly increased the activities of 6-phosphofructokinase, pyruvate dehydrogenase, and succinate dehydrogenase regardless of the carbohydrate level. The level of malondialdehyde decreased significantly with increased α-LA supplementation; in contrast, the levels of superoxide dismutase, glutathione peroxidase, and glutathione were significantly higher in prawns fed 700 or 1400 mg/kg α-LA when compare with those fed no α-LA supplement. Dietary α-LA did not affect the overall morphology of the hepatopancreas, but 1400 mg/kg α-LA reduced the number of B (blister-like) cells. Thus, 700 mg/kg α-LA supplementation under either dietary carbohydrate level could improve the antioxidant status, carbohydrate metabolism, and morphology of the hepatopancreas in juveniles of *M. nipponense*. We conclude that α-LA supplementation can be used to improve the efficiency of carbohydrate utilization when a 30% carbohydrate diet is used for this prawn.

1. Introduction

Carbohydrate is an important and cheap energy source in aquatic animal diets. It plays important roles in physiological metabolism, such as citric acid cycle, gluconeogenesis, chitin synthesis, and the formation of steroids and fatty acids in animals (Wigglesworth and Griffith, 1994).

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Appropriate increases in the carbohydrate level in aquatic animal diets can spare dietary protein, save diet cost, decrease the excretion of ammonia and nitrogen, and alleviate water pollution (Alava and Pascual, 1987; Rosas et al., 2000). However, aquatic animals have a limited ability to utilize carbohydrate (Holme et al., 2009; Shiau and Huang, 1990). Consequently, feeding aquatic animals with high carbohydrate diets usually cause metabolic disorders (Ma et al., 2020; Song et al., 2018). Therefore, improving the utilization efficiency of high carbohydrate is important for sustainable aquaculture development.

Alpha-lipoic acid (α-LA) is a cofactor of key enzymes (pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase) involved in glucose metabolism, which catalyzes the process of oxidative decarboxylation and plays an important role in energy metabolism of carbohydrate (Lobato et al., 2018). In higher animals, α-LA improved the ability of glucose uptake, utilization and insulin sensitivity (Lee et al., 2005) and prevented the development of type 2 diabetes (Song et al., 2004). Dietary α-LA also limited the adverse syndrome caused by diabetes (Kowluru and Odenbach, 2004; Shay et al., 2009). Similarly, *in vitro* studies found that α-LA mainly participated in aerobic oxidation of mitochondria in cells and promoted tricarboxylic acid cycle (TCA) metabolism of carbohydrate (Ikuta et al., 2017). Moreover, α-LA has been indicated to inhibit anaerobic glycolysis of carbohydrate in various studies (Schwarts et al., 2017; Ikuta et al., 2017). Apart from carbohydrate metabolic influence, α-LA is also a powerful non enzymatic antioxidant, scavenging free radicals and chelating heavy metal ions and thus maintaining the normal oxidation-antioxidant level in the body (Packer et al., 1995). This role has been confirmed in many studies for higher or lower animals (Somi et al., 2011; Guo et al., 2014; Monserrat et al., 2008; Shi et al., 2017; Xu et al., 2018, 2019).

Studies involving dietary α-LA on aquatic animals mainly focused on its effects on growth, oxidative stress and lipid metabolism. In such studies, dietary α-LA positively modulated oxidative stress under hypoxia/reoxygenation conditions and was effective as a supplement against damage induced by hypoxia in *Litopenaeus vannamei* (Lobato et al., 2018). α-LA alleviated oxidative damage caused by lipid deposition and improved the health status of Chinese mitten crab *Eriocheir sinensis* (Xu et al., 2018). However, studies on the regulation of α-LA on carbohydrate metabolism in aquatic animals are currently limited. The only study available in carp (*Cyprinus carpio*) showed that α-LA regulated the metabolism of lipid, protein and carbohydrate, and improved the utilization efficiency of carbohydrate (Santos et al., 2016). As mentioned above, α-LA can regulate glucose metabolism by regulating insulin secretion (Lee et al., 2005), so the effect of α-LA on glucose metabolism of fish might be closely related to the secretion of this hormone. However, the hormones involved in carbohydrate regulation in crustaceans are different from those in fish (Wang et al., 2016). More likely, the utilization and effects of α-LA might be different between fish and prawns, a knowledge currently missing in the scientific literature.

The freshwater prawn, *Macrobrachium nipponense,* has become one of the main economic freshwater prawns in China due to its fast growth in short culture period (Ma et al., 2011). However, studies on the nutritional aspects of *M. nipponense* are currently limited. Previous studies concentrated on carbohydrate source and its appropriate level in *M. nipponense* (Ding et al., 2017; Kong et al., 2019) and replacement of fishmeal by soybean and cotton seed meals (Huang et al., 2018). In these studies, Ding et al. (2017) and Kong et al. (2019) indicated that *M. nipponense* has some moderate metabolic adaptations for using dietary carbohydrate and fishmeal can be replaced in its diet by using soybean and cotton seed meals (Huang et al., 2018). Finding appropriate approaches to improve the utilization efficiency of carbohydrate in *M. nipponense* offers opportunity for improved health of the species, reduced energy sources from other nutrients for its sustainable production. It should be noted that although α-LA has not been reported to regulate carbohydrate metabolism in crustaceans, appropriate α-LA supplement to regulate energy metabolism has been studied (Xu et al., 2018). Therefore, this study explored the effects of α-LA on growth performance, antioxidant status, hepatopancreas morphology and carbohydrate metabolism in *M. nipponense* fed two dietary carbohydrate levels.

2. Materials and methods

2.1. Experimental diets

Six experimental diets for *M. nipponense* were formulated with casein and fish meal as the protein sources, fish oil and soybean oil as the lipid sources, and corn starch as the carbohydrate source, at two carbohydrate levels (15% and 30%), each supplemented with three concentrations of α-LA (0, 700, and 1400 mg/kg). The concentrations of α-LA used in this study was based on our previous studies (unpublished data). The three low-carbohydrate diets (15% carbohydrate) were LCLA1, LCLA2, and LCLA3; the three high-carbohydrate diets (30% carbohydrate) were HCLA1, HCLA2, and HCLA3 group (Table 1). After 8 weeks of feeding, the growth performance, antioxidant indexes, and activities of key enzymes of the glycolysis pathway and citric acid cycle of the prawns were evaluated.

All dry ingredients of the diets were passed through a 212-μm mesh before being thoroughly mixed with distilled water. This mixture was extruded through a 1.5-mm die with a pelleting machine, and then dried in a forced-air oven at 40 °C and stored at −20 °C until use.

2.2. Experimental animals and feeding trial

Prawns, with normal-looking and without evidence of any disease, were purchased from Huzhou Bangda Ecological Agriculture Co. Ltd. in Zhejiang Province, China. Prior to the feeding trial, prawns were fed a commercial diet (39% protein) for 1 week to acclimate to the experimental conditions. At the beginning of the experiment, healthy prawns (initial weight 0.050 ± 0.003 g) were randomly distributed in 30 tanks (six groups) of 300-L volume. Five replicates were used for each group, and each tank held 50 prawns. Prawns were fed twice daily to apparent satiation, and the feeding trial lasted 8 weeks. To reduce animal cannibalization, some nylon fishing nets were placed as artificial shelter in each tank. During the experimental period, water-quality parameters were monitored to maintain a temperature of 25–28 °C, dissolved oxygen of >6.5 mg L^{-1}, and ammonia and nitrate levels of <0.1 mg L^{-1}, under a natural daylight cycle. One-third of the water in each tank was replaced daily.

2.3. Hepatopancreas sample collection

Prawns were deprived of food for 24 h prior to sampling. The prawns in each tank were counted and weighed to determine growth performance. The hepatopancreas were dissected from the cephalothorax; some of these were fixed in Bouin's solution for 24 h before histological analysis, and some were stored at −80 °C for the analyses of enzyme activities.

2.4. Growth performance of prawns and proximate composition of the diets

Weight gain, specific growth rate, and survival rate of the *M. nipponense* were calculated as in the earlier study (Ding et al., 2017). Determinations of the moisture, crude lipid, and crude protein contents of the diets were conducted using standard procedures (AOAC, 2005). Moisture content was determined by oven-drying at 105 °C to a constant weight. Crude protein was measured by the combustion method, using an FP-2000 nitrogen analyzer (Leco Corp., St. Joseph, MI, USA). Crude lipid was determined by ether extraction using a Soxtec System (Soxtec Avanti 2055; Foss Tecator, Höganäs, Sweden).

Table 1
Ingredients and proximate compositions of the experimental diets (%, dry weight).

Ingredients	15% carbohydrate			30% carbohydrate		
	0 LCLA1	700 LCLA2	1400 LCLA3	0 HCLA1	700 HCLA2	1400 HCLA3
Casein	33	33	33	33	33	33
Fish meal	20	20	20	20	20	20
corn starch	15	15	15	30	30	30
α-lipoic acid	0	0.07	0.14	0	0.07	0.14
Fish oil: Soybean oil (2:1)	3.5	3.5	3.5	3.5	3.5	3.5
Soybean lecithin	0.5	0.5	0.5	0.5	0.5	0.5
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[a]	2	2	2	2	2	2
Mineral premix[b]	3	3	3	3	3	3
Attractant[c]	3	3	3	3	3	3
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Cellulose	20	19.93	19.86	5	4.93	4.86
Sodium carboxymethylcellulose	2	2	2	2	2	2
Total	100	100	100	100	100	100
Proximate composition						
Dry matter	82.26	82.07	82.37	81.45	81.99	82.20
Crude protein	38.51	38.65	38.51	38.51	38.29	38.25
Crude lipid	7.26	7.62	7.73	7.21	7.62	7.69
Crude ash	11.24	11.13	10.94	11.09	11.02	12.66

[a] Vitamin mixture (mg 100 g^{-1}mixture): vitamin A 420000 IU, vitamin C 6000 mg, α-tocopherol acetate 2000 mg, vitamin D_3 120,000 IU, vitamin K 1000 mg, vitamin B_1 750 mg, vitamin B_2 1000 mg, vitamin B_6 1600 mg, vitamin B_{12} 2 mg, niacin 5000 mg, folic acid 400 mg, inositol 6000 mg, biotin 10 mg, calcium pantothenic 3500 mg.

[b] Mineral mixture (mg g^{-1} mixture): KCL 28; $MgSO_4 \cdot 7\ H_2O$ 100; NaH_2PO_4 215; KH_2PO_4 100; $Ca(H_2PO_4) \cdot 2\ H_2O$ 265; $CaCO_3$ 105; $C_6H_{10}CaO_6 \cdot 5\ H_2O$ 165; $FeC_6H_5O_7 \cdot 5\ H_2O$ 12; $ZnSO_4 \cdot 7\ H_2O$ 4.76; $MnSO_4 \cdot H_2O$ 1.07; $AlCL_3 \cdot 6\ H_2O$ 0.15;$CuCl_2 \cdot 2\ H_2O$ 0.24; $CoCl_2 \cdot 6\ H_2O$ 1.4; KI 0.23; α-cellulose 2.15.

[c] Attractant: alanine 0.6%, glycine 0.6%, glutamic acid 0.6%, betaine 1.2%.

2.5. Carbohydrate metabolism enzyme activities

The hepatopancreas enzyme activities of hexokinase (HK; EC 2.7.1.1), pyruvate kinase (PK; EC 2.7.1.40), 6-phosphofructokinase (PFK; EC 2.7.1.11), pyruvate dehydrogenase (PDH; EC 4.1.1.1), and succinate dehydrogenase (SDH; EC 1.3.5.1) were determined by using kits purchased from Suzhou Comin Biotechnology Co. Ltd. (Suzhou, China) based on manufacturer's instructions. The idea of making the determinations with these techniques was as described by Li et al. (2013) and Wang et al. (2017), and the absorbance values were read with a Multiskan Spectrum purchased from Thermo (Multiskan GO 1510).

2.6. Morphology of the hepatopancreas and antioxidant enzyme activities

For analysis of the tissue morphology, the fixed hepatopancreas of *M. nipponense* were dehydrated in ascending concentrations of alcohol. Dehydrated tissues were embedded in paraffin and the blocks were cut into continuous 5-μm sections. To reveal changes in the hepatopancreas, the 5-μm sections were stained with hematoxylin and eosin, and then examined with CaseViewer software (3DHISTECH Ltd., Budapest, Hungary).

To determine the antioxidant enzyme activities, samples of hepatopancreas were homogenized with 0.86% (*w/v*) precooled saline solution (0.86 g of sodium chloride was added to 100 mL distilled water, pH 7.0). The homogenates were centrifuged at 1500*g* (5415R, Eppendorf, Germany) for 20 min and the supernatants were collected. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, and reduced glutathione (GSH) and the malondialdehyde (MDA) content in the hepatopancreas were determined using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The idea of making the determinations with these techniques was as described by Liu et al. (2020), and Thermo Multiskan Spectrum was used to perform the absorbance readings.

2.7. Statistical analyses

Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Data are reported as mean ± standard deviation (SD). Data were analyzed by two-way analysis of variance (ANOVA) to determine whether there was any significant interaction between the concentration of dietary α-LA and the carbohydrate level. At the same carbohydrate level, one-way analysis of variance (ANOVA) was used to assess differences among groups fed diets with different concentrations of α-LA, after tests of normality and homogeneity of variance. When the means of each treatment significantly differed, Turkey's multiple-range test was used to compare means among these treatments. For the same α-LA concentration, an independent-samples *t*-test was used to determine whether there were statistically significant differences between groups fed the low-carbohydrate and high-carbohydrate diets. A *P*-value less than 0.05 was considered significant; $P < 0.01$ was considered extremely significant.

3. Results

3.1. Growth performance of prawns

The prawns fed on the 1400 mg/kg LA diet decreased significantly the weight gain (Fig. 1A) and specific growth rate (Fig. 1B) compared to those fed on 0 and 700 mg/kg α-LA diets ($P < 0.05$). Feeding the prawns with α-LA at high carbohydrate diet had no significant effect on the weight gain and specific growth rate. The prawns fed the 700 mg/kg LA at low and high carbohydrate diets increased significantly the survival rate than those fed on 0 and 1400 mg/kg α-LA diets ($P < 0.05$) (Fig. 1C). Comparing groups given the same α-LA supplementation, survival was significantly less in groups fed a high-carbohydrate diet ($P < 0.05$). The carbohydrate and α-LA levels interacted significantly to affect weight gain and specific growth rate but not survival rate.

3.2. Activities of enzymes of carbohydrate metabolism

Significant interactions between the carbohydrate level and α-LA

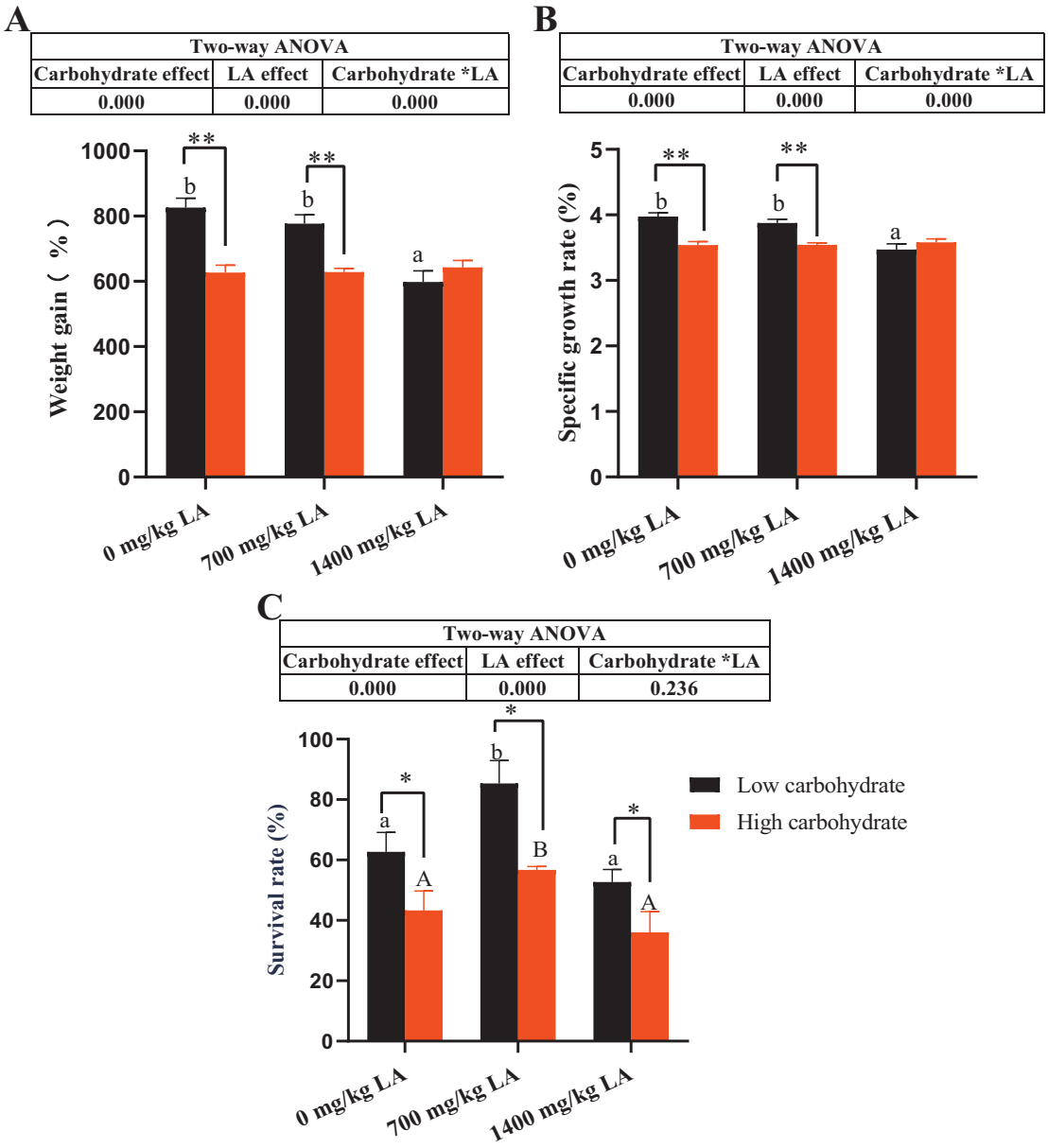


Fig. 1. Growth performance of freshwater prawns *Macrobrachium nipponense* in relation to the concentration of α-lipoic acid supplementation under two dietary carbohydrate levels (low = 15%; high = 30%). Data are presented as mean ± SD. Columns with different superscripts are significantly different at $P < 0.05$: lowercase denotes significant differences among groups fed the low-carbohydrate diet; capital letters denote significant differences among groups fed the high-carbohydrate diet.

concentration were found for the activities of PFK, PK and PDH ($P < 0.01$) (Fig. 2). The activities of HK and PK were highest in prawns fed l400 mg/kg α-LA, in both the low- and high-carbohydrate groups. Among the low-carbohydrate groups, PFK activity was significantly higher in prawns fed 700 mg/kg α-LA compared with the other two concentrations; among the high-carbohydrate groups, the PFK activity was highest in prawns fed 1400 mg/kg α-LA ($P < 0.05$). Supplementation with 700 or 1400 mg/kg α-LA significantly increased the PDH and SDH activities regardless of the dietary carbohydrate level ($P < 0.05$). Thus, irrespective of the concentration of α-LA supplementation, a high-carbohydrate diet significantly induced the activities of HK, PFK, and PDH when compared with the effects of a low-carbohydrate diet ($P < 0.05$). However, the activity of PK was significantly higher in prawns fed a high-carbohydrate diet, but only at 1400 mg/kg α-LA ($P < 0.05$).

3.3. MDA content and antioxidant enzyme activities

Two-factor analysis showed that the concentration of α-LA had main effects on MDA, GSH, and SOD ($P < 0.01$). In the low-carbohydrate groups, the MDA level of prawns decreased significantly with an increase in dietary α-LA ($P < 0.05$); SOD activity was significantly higher in prawns fed 700 mg/kg α-LA than in prawns fed 0 or 1400 mg/kg α-LA ($P < 0.05$); and a higher GSH level was also observed in prawns fed 700 mg/kg α-LA. In the high-carbohydrate groups, the MDA level likewise decreased significantly with an increase in dietary α-LA ($P < 0.05$); SOD activity was significantly higher in prawns fed 1400 mg/kg α-LA than in prawns fed 0 or 700 mg/kg α-LA ($P < 0.05$); GSH-Px activity was significantly higher in prawns fed 700 or 1400 mg/kg α-LA than that of prawns fed no α-LA supplement ($P < 0.05$); and the highest GSH level

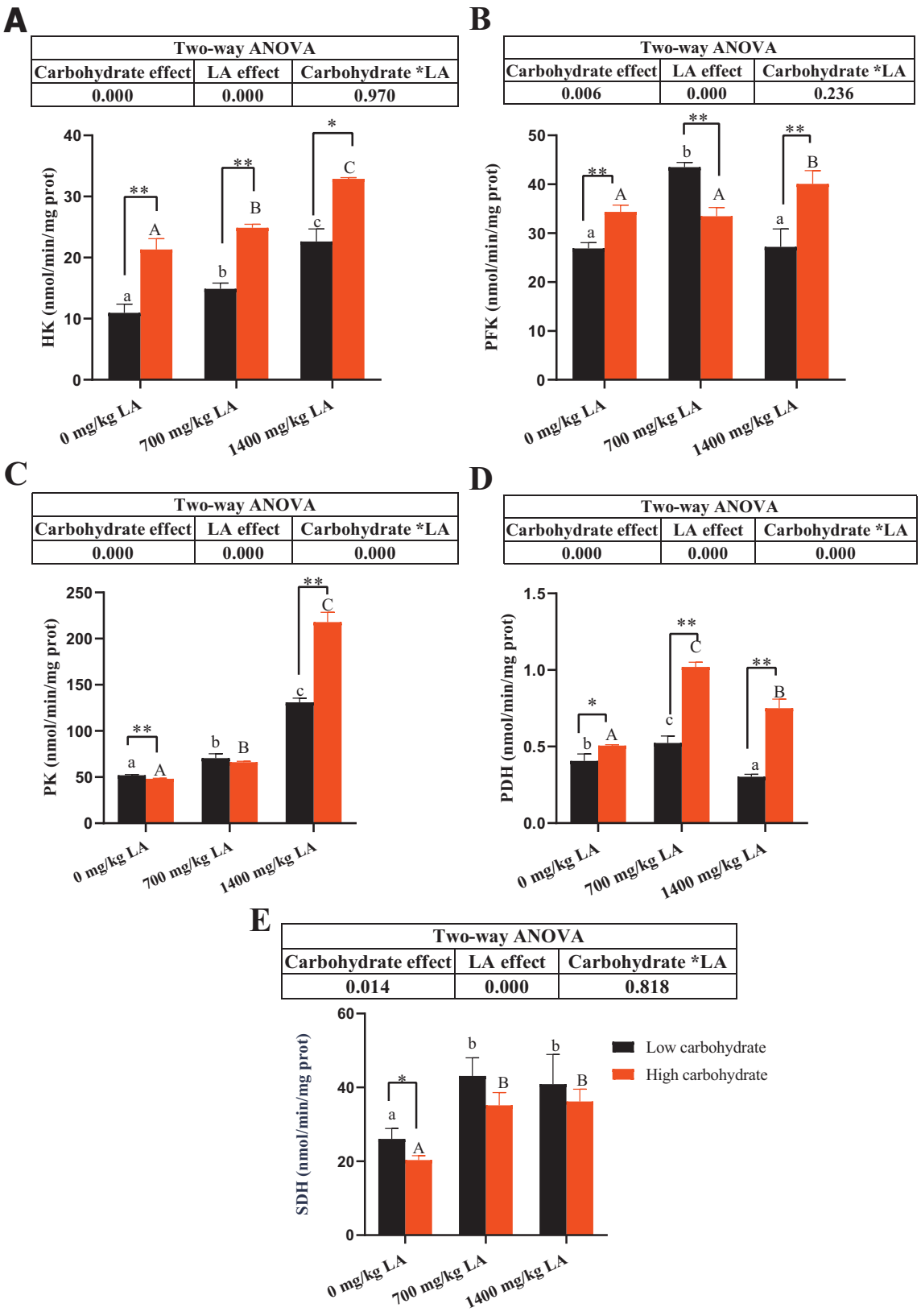


Fig. 2. Carbohydrate metabolism enzyme activities in the hepatopancreas of freshwater prawns *Macrobrachium nipponense* in relation to the concentration of α-lipoic acid supplementation under two dietary carbohydrate levels. Data are presented as mean ± SD. Columns with different superscripts are significantly different at $P < 0.05$: lowercase denotes significant differences among groups fed the low-carbohydrate diet; capital letters denote significant differences among groups fed the high-carbohydrate diet. HK, hexokinase; PFK, 6-phosphofructokinase; PK, pyruvate kinase; PDH, pyruvate dehydrogenase; SDH, succinate dehydrogenase.

was obtained in prawns fed 1400 mg/kg α-LA. The dietary carbohydrate level mainly affected the levels of MDA and GSH ($P < 0.01$). There was significant interaction between the carbohydrate and α-LA levels and lipid oxidation and antioxidant enzyme activities ($P < 0.01$) (Fig. 3).

3.4. Hepatopancreas morphology

The hepatopancreas of the prawns fed low (Fig. 4a) and high carbohydrate (Fig. 4d) supplemented with 0 mg/kg LA had less blister cells (B-cells) than those fed low (Fig. 4b) and high carbohydrate (Fig. 4e) diets supplemented with 700 mg/kg LA, respectively. Feeding the prawns with low (Fig. 4c) and high carbohydrate (Fig. 4f) diets supplemented with 1400 mg/kg LA decreased the number of B-cells in the hepatopancreas.

4. Discussion

In the present study, we found that dietary α-LA decreased the growth rate of *M. nipponense* fed a low-carbohydrate diet. Significant growth-inhibition effects of lipoic acid were also observed in juvenile grass carp *Ctenopharyngodon idellus* (Huang et al., 2019) and Plata pompano *Trachinotus marginatus* (Kütter et al., 2012). Growth inhibition from dietary α-LA in animals occurs because α-LA can play a substantial role in the regulation of food intake by suppressing appetite and elevating energy metabolism (Kim et al., 2008; Seo et al., 2012). In the present study, the prawns had reduced their appetite and food intake after feeding the diets supplemented with high α-LA, which indicated low concentration of α-LA may be more adequate for the growth of prawns. The poor appetite and decreased body weight may be attributed to the changes of peripheral appetite regulating factors as described in grass carp (Huang et al., 2019). The high levels of α-LA may also induce cellular stress that increased the energy spend of *M. nipponense*, thereby affecting the growth of the prawns. However, some studies have found that a suitable concentration of dietary α-LA promoted better growth. For example, Xu et al. (2019) found that α-LA supplementation at 700 or 1400 mg/kg significantly improved the growth performance of juvenile Chinese mitten crab *Eriocheir sinensis*. Zhang et al. (2010) similarly found that weight gain in juvenile abalone *Haliotis discus hannai*

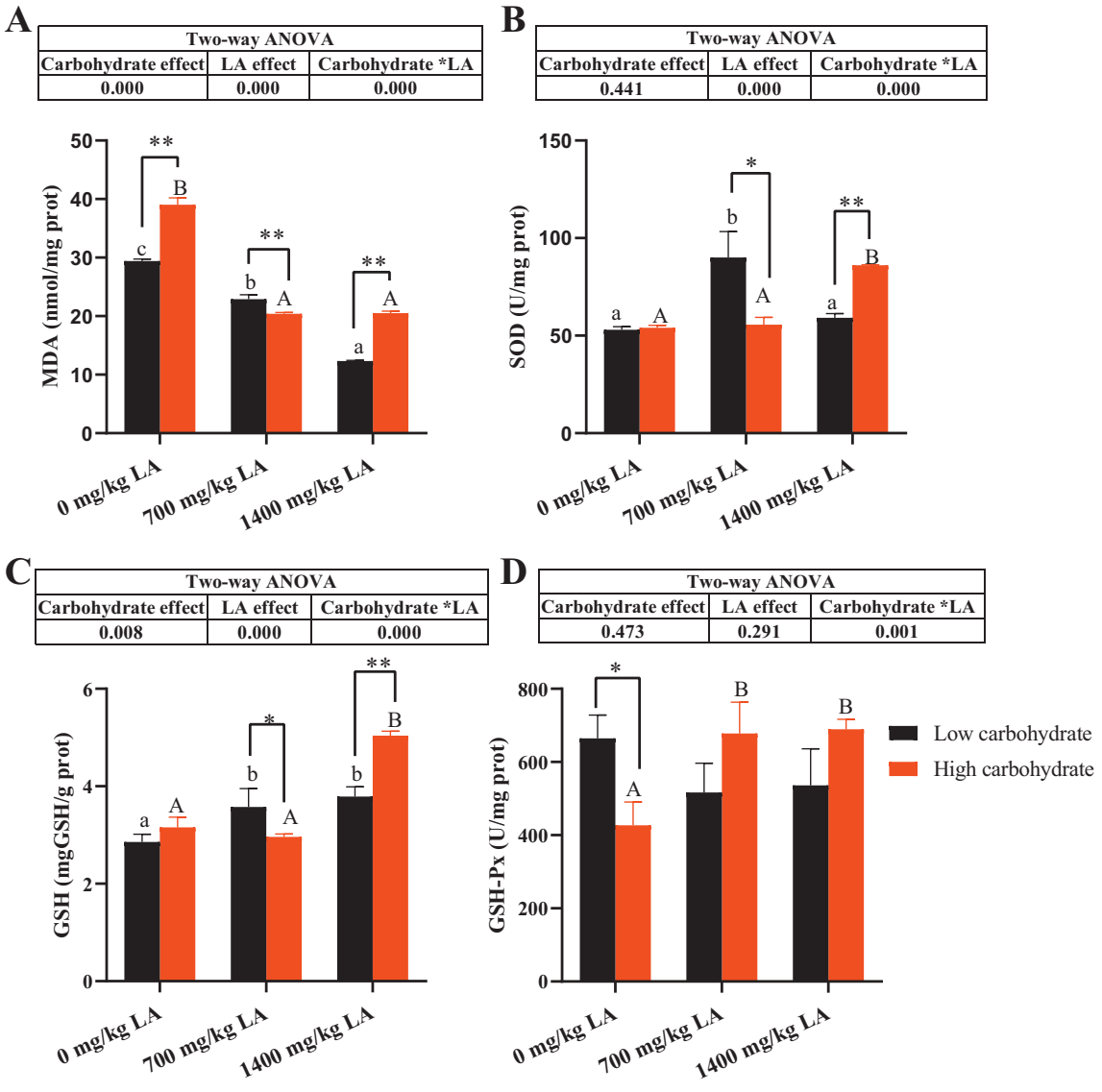


Fig. 3. Antioxidant capacity in the hepatopancreas of freshwater prawns *Macrobrachium nipponense* in relation to the concentration of α-lipoic acid supplementation under two dietary carbohydrate levels. Data are presented as mean ± SD. Columns with different superscripts are significantly different at $P < 0.05$: lowercase denotes significant differences among groups fed the low-carbohydrate diet; capital letters denote significant differences among groups fed the high-carbohydrate diet. MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; GSH-Px, glutathione peroxidase.

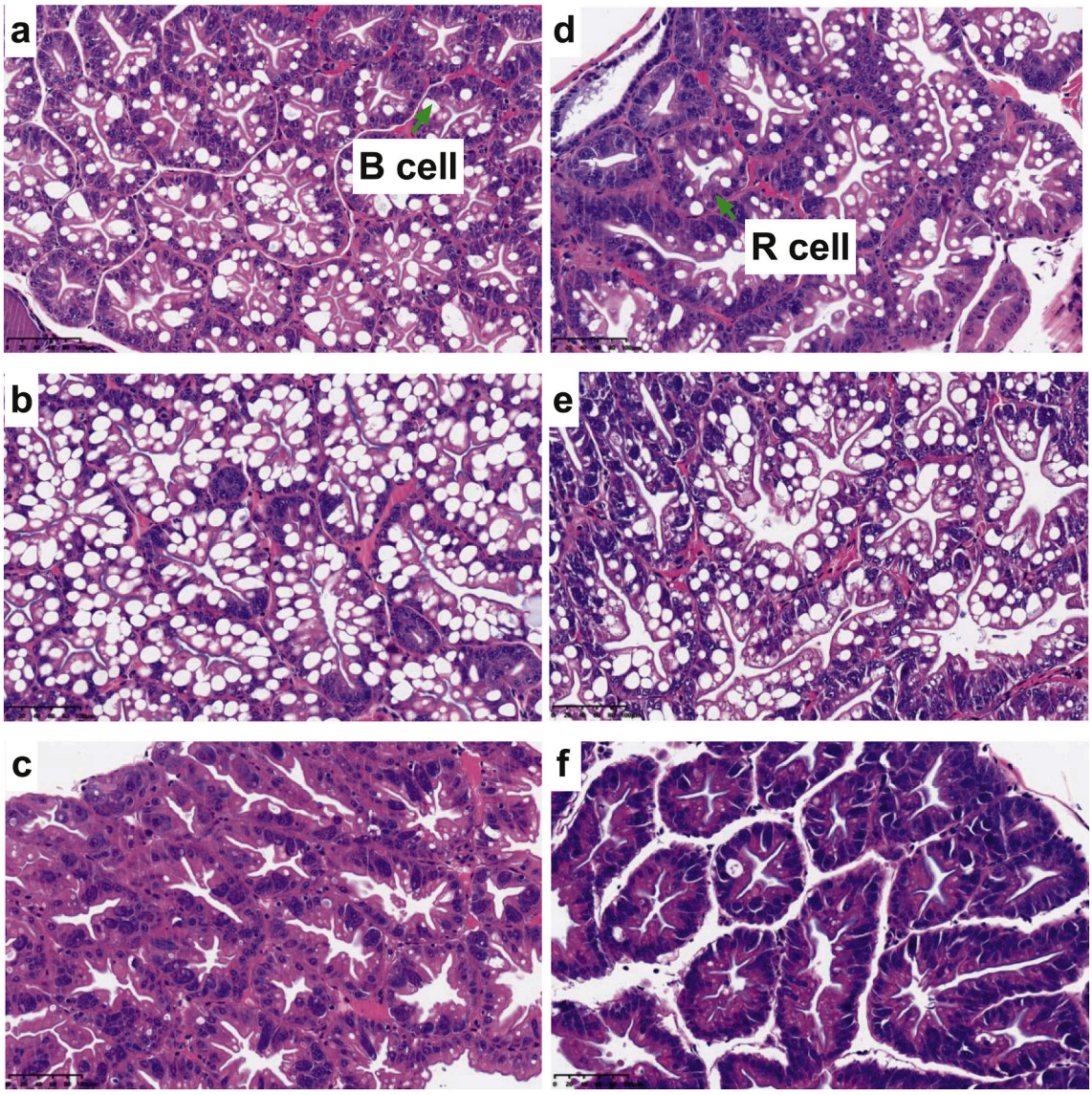


Fig. 4. The hepatopancreas morphology of the freshwater prawn, *Macrobrachium nipponense* fed on low and high carbohydrate levels supplemented with α-lipoic acid. Hepatopancreas from prawns fed on low carbohydrate supplemented with 0, 700 and 1400 mg/kg are represented by a, b and c, respectively. Hepatopancreas from prawns fed on high carbohydrate supplemented with 0, 700 and 1400 mg/kg are represented by d, e and f, respectively. R-cell represents resorptive cell and B-cell represents blister cell.

increased with dietary α-LA, and the rate peaked at 800 mg/kg dietary α-LA. In general, these results suggest that the effect of α-LA on growth performance depend on species and dose used. Interestingly, Santos et al. (2016) found 1000 mg/kg α-LA had positive effects on common carp growth fed with glucose diets, but decreased weight gain on fed with starch diets. In our study, we also found that feeding the prawns with α-LA at high carbohydrate diet had no significant effect on the growth of *M. nipponense*. That is to say, dietary ingredients can affect growth regulation of α-LA to animals.

A high-carbohydrate diet inhibited the growth of *M. nipponense*, which corresponds to the results of an earlier study of this species (Ding et al., 2017). When the dietary carbohydrate level exceeds the optimum, the excess may decrease the ability of the animal to digest, absorb and assimilate the carbohydrate ingredient (Xiao et al., 2014). Furthermore, excess dietary carbohydrate cannot be used as an energy source, led to a low protein efficiency and was converted to lipid accumulated in the body (Rosas et al., 2000; Zhan et al., 2020). Interestingly, α-LA supplementation did not decrease the growth rate of prawns fed the high (30%)-carbohydrate diet in the present study. Body weight is determined by the balance between food intake and energy expenditure (Kim et al., 2004). Although dietary α-LA increased energy expenditure, the energy provided by a high-carbohydrate feed likely countered the enhanced expenditure. This can be seen from the significantly elevated activities of the enzymes involved in glycolysis and the respiratory chain assessed in prawns fed an α-LA supplement. Hexokinase (HK), pyruvate kinase (PK), and 6-phosphofructokinase (PFK) are rate-limiting enzymes in glycolysis (Nelson and Cox, 2004), so the increased activities of these enzymes indicated glycolysis was upregulated by dietary carbohydrate or α-LA, and more pyruvate was later provided for energy metabolism. Pyruvate dehydrogenase complex (PDH) is a mitochondrial matrix multienzyme complex that provides the link between glycolysis and the TCA cycle by catalyzing the conversion of pyruvate into acetyl-CoA (Kantor et al., 2001). Increased pyruvate oxidation and PDH complex activation were also found in rat hepatocytes after therapeutically relevant doses of α-LA for 3 h (Walgren et al., 2004). The increased PDH indicated lactate formation was prevented and more flow of acetyl-CoA into the citric acid cycle for aerobic metabolism (Feuerecker et al., 2012). This also can be confirmed from the increased activity of

succinate dehydrogenase (SDH) with dietary α-LA, a key enzyme of the respiratory chain which is located in the inner mitochondrial membrane, and it is reported to be of critical functional importance when the energy request is high (Tondo et al., 2015). Moreover, a high-carbohydrate diet significantly decreased SDH activity yet the higher concentrations of α-LA counteracted the negative effect from high carbohydrate intake. Whereas a high-carbohydrate diet caused slower growth and decreased respiratory chain activity in this freshwater prawn, high α-LA supplementation counteracted the negative effects. The data indicate that lipoic acid stimulated glucose metabolism, which is supported by numerous investigators who report that α-LA in the medium stimulated glucose metabolism in different cell models (Ikuta et al., 2017; Schwarts et al., 2017).

In addition to regulating glucose metabolism, α-LA also affected the survival, antioxidant capacity, and histological morphology of the hepatopancreas of *M. nipponense* under both low- and high-dietary carbohydrate levels. When no α-LA was added, the survival rate decreased among prawns fed high carbohydrate, which might be related to stress induced by the high glucose load. This can be verified from the higher MDA content, a widely used biomarker for lipid peroxidation (Sies, 1991), in the high-carbohydrate groups. However, with supplementation at 700 mg/kg α-LA, the stress was alleviated and the survival rate of prawns increased, which might have been an outcome of improved antioxidant capacity (except for increased glucose metabolism, discussed above) from the α-LA supplementation. GSH is a non-enzymatic antioxidant molecule, which can directly or indirectly scavenge ROS (Espinosa-Diez et al., 2015). Consequently, the supplementation of the carbohydrate diet with 700 mg/kg LA decreased the MDA content accompanied by a significant increase of GSH level in the hepatopancreas of prawns regardless carbohydrate level, due to increased antioxidant capacity in prawns. SOD is the first enzyme to respond against reactive oxygen species (ROS), which catalyzes dismutation of superoxide radicals to hydrogen peroxide, and hydrogen peroxide is then transformed into water by GSH-Px (Livingstone, 2003). The increased activities of SOD and GSH-Px indicated ROS and hydrogen peroxide were generated for the prawns. However, from the decreased MDA content after α-LA supplementation, it seems that the α-LA indued some stress but the α-LA was enough to cope with this situation. Similarly, appropriate addition of α-LA in the diet of *E. sinensis* could activate the enzymatic and non-enzymatic antioxidant systems and improve the antioxidant capacity of the crabs (Xu et al., 2018); furthermore, supplementation with α-LA in feed for *Litopenaeus vannamei* positively modulated lipid peroxidation and antioxidant capacity upon exposure to hypoxia and reoxygenation (Lobato et al., 2018). These results suggest that, supplementing prawn diet with 700 mg/kg LA reduces oxidative stress by increasing antioxidant capacity.

Interestingly, the highest concentration of α-LA (1400 mg/kg) likewise improved the activities of antioxidant enzymes in *M. nipponense*, but not their survival rate. It is reported that α-lipoic acid could exert both pro- and antioxidant effects depending on the underlying physiologic and metabolic state of animals (Bhatti et al., 2005). α-lipoic acid and its major metabolite have also been shown to stimulate superoxide anion production in rat liver mitochondria and submitochondrial particles and in cultured 3 T3-L1 adipocytes in response to high glucose stimuli (Moini et al., 2002). So, it cannot exclude the possibility that 1400 mg/kg α-LA may act as a pro-oxidant, causing deleterious effects to the health of prawns and its survival rate.

Furthermore, the prawns fed on diet supplemented with 1400 mg/kg LA had reduced number of B cells. B (blister-like) cells are relatively large cells responsible for the synthesis of digestive enzymes (Pourmozaffar et al., 2019). An increase in hepatopancreatic B cells was observed at low salinities in L. *vannamei* (Li et al., 2008). Such an increase was suggested to facilitate the synthesis and excretion of the digestive enzymes, which enabled the shrimp to derive more energy from the feed (Li et al., 2008). Therefore, the increase of hepatopancreatic B cells in prawns fed 700 mg/kg α-LA indicated more digestive enzymes were synthesized and excreted. However, 1400 mg/kg α-LA decreased synthesis of the digestive enzymes in *M. nipponense*, which resulted in less energy obtained from feed. The less energy obtained and more energy expenditure in prawns fed 1400 mg/kg α-LA resulted in energy imbalance. This also might be one of the reasons for low survival rate of the prawns fed the diet supplemented with 1400 mg/kg LA as reported previously in crabs (Lu et al., 2016). Reduced B cells in hepatopancreas of L. *vannamei* fed on organic acid was suggested as a reason for possible reduced reliance on digestive enzymes in the digestion process (Romano et al., 2015). Whether high levels of α-LA reduced reliance on digestive enzymes in *M. nipponense* needs further investigation.

5. Conclusions

In conclusion, the growth of *M. nipponense* was inhibited by dietaryα-LA at low carbohydrate level, but not at high carbohydrate level. Dietary α-LA promoted glycolysis in the TCA cycle and aerobic oxidation of mitochondria, improving carbohydrate metabolism regardless of dietary carbohydrate levels. Supplementing the carbohydrate diets with 700 mg/kg α-LA reduced oxidative stress by increasing antioxidant capacity. In addition, dietary 1400 mg/kg α-LA reduced the number of B cells, resulting in loss of energy homeostasis and low survival rate. Thus, supplementing the diets of prawns fed carbohydrate with 700 mg/kg α-LA improves antioxidant status, carbohydrate metabolism and the structure of hepatopancreas. We recommend supplementing α-LA to the diet of *M. nipponense* when 30% carbohydrate is used in order to improve feed utilization efficiency.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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