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**Effects of  $\alpha$ -lipoic acid on growth performance, body composition, antioxidant status and lipid catabolism of juvenile Chinese mitten crab *Eriocheir sinensis* fed different lipid percentage**

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**Abstract**

This study evaluates the effects of dietary lipid percentage (7% and 13%) on growth performance, body composition, antioxidative status and hepatopancreas lipid catabolism of Chinese mitten crab *Eriocheir sinensis*. Each lipid diet was supplemented with three concentrations of  $\alpha$ -lipoic acid at 0, 700 and 1400 mg/kg, and fed to *E. sinensis* juveniles for eight weeks. The weight gain and specific growth rate of crabs fed the diets supplemented with  $\alpha$ -LA were significantly higher than those fed the control diet without  $\alpha$ -LA, regardless of dietary lipid percentage. The  $\alpha$ -LA significantly increased lipid accumulation in the whole body and hepatopancreas in a dose-dependent manner. Crabs fed 13% lipid showed a significantly higher hepatosomatic index than those fed 7% lipid. The mRNA expressions of triacylglycerol lipase and intracellular lipase increased with the increase of  $\alpha$ -LA concentration in crabs fed 7% lipid. No significant difference was found in the CPT-1 mRNA expression among all treatments. The  $\alpha$ -LA supplementation at 1400 mg/kg significantly improved oxidative stress due to lipid accumulation in the hepatopancreas of crabs fed 7% lipid as indicated by the high activity of superoxide dismutase and glutathione peroxidase and a low level of malondialdehyde. The diet with 13% lipid increased the lipid content in the hepatopancreas but suppressed glutathione peroxidase. Meanwhile, the total antioxidant capacity increased with the increase of  $\alpha$ -LA concentration in crabs fed 13% lipid. This study indicates that  $\alpha$ -LA supplementation can improve growth performance and accelerate lipid accumulation in the hepatopancreas by increasing lipid utilization efficiency. Furthermore,  $\alpha$ -LA can relieve hepatopancreas oxidative damage induced by lipid accumulation and improve the health of *E. sinensis*.

**Keywords:** *Eriocheir sinensis*,  $\alpha$ -lipoic acid, growth performance, body composition, antioxidant status, lipid catabolism

## 1. Introduction

The  $\alpha$ -lipoic acid ( $\alpha$ -LA) was first identified by Lester Reed in 1951 and has been gained wide attention due to its various biological functions (Reed et al., 1951). The  $\alpha$ -LA can be synthesized *de novo* in most animals, plants and microorganisms and it is a cofactor of different complexes in the oxidative decarboxylation enzyme of the Krebs cycle, i.e., pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase (Bast and Haenen, 2003; Trattner et al., 2007). Previous studies have shown that  $\alpha$ -LA and its reduced form as dihydrolipoic acid (DHLA) have potent antioxidant capacity in both aqueous and lipidic environments because of the amphiphilic property to cross the cell membrane and the blood brain barrier (Gonzalez-Perez and Gonzalez-Castaneda, 2006; Yoshikawa et al., 1996). Appropriate supplementation of  $\alpha$ -LA in the diet can significantly improve growth performance, survival and antioxidant status in abalone *Haliotis discus hannai* (Zhang et al., 2010) and hybrid tilapia *Oreochromis niloticus*  $\times$  *Oreochromis aureus* (Xiong et al., 2012) due to its ability to reduce reactive oxygen species (ROS) (Bast and Haenen, 2003) and regenerate other antioxidants such as vitamin C, vitamin E, glutathione, coenzyme Q10 and ubiquinone (Han et al., 1997; Park et al., 2006; Trattner et al., 2007). Moreover, due to its capacity to chelate metals as a neuroprotector and easy absorption from a diet,  $\alpha$ -LA can also protect against metallic pollutants and hypoxia stress in white shrimp *Litopenaeus vannamei* (Lobato et al., 2013; Martins et al., 2014) and microcystin-induced toxicosis in common carp *Cyprinus carpio* (Amado et al., 2011). In the past, the role of  $\alpha$ -LA has been studied in many poultry (Guo et al., 2014; Wang, 2015; Yasin et al., 2012) and fish species (Enamorado et al., 2015; Kütter et al., 2013; Kütter et al., 2012; Monserrat et al., 2008; Sevgiler et al., 2011). However, excessive  $\alpha$ -LA supplementation can negatively impact animal growth and antioxidative capacity (Xiong et al., 2012; Zhang et al., 2010), therefore the appropriate dose for economically important species in aquaculture should be determined. Despite being a universal antioxidant, our understanding of the effect of  $\alpha$ -LA on growth performance and survival in crustacean is limited.

In addition to the role of powerful antioxidant,  $\alpha$ -LA can regulate lipid

metabolism (Jayanthi and Varalakshmi, 1992) and is used to treat diabetes complications, cardiovascular disease, hypertension, obesity and Alzheimer's disease (Kim et al., 2004; Maczurek et al., 2008; Shay et al., 2009). In rats,  $\alpha$ -LA can suppress appetite, decrease body weight (Kim et al., 2004) and activate Sirtuin 1 (SIRT1) and AMP-activated protein kinase (AMPK), which are highly effective for reducing intracellular triacylglycerol accumulation of C<sub>2</sub>C<sub>12</sub> myotubes by increasing adipose triacylglycerol lipase (ATGL) and suppressing fatty acid synthase (FAS) (Chen et al., 2012). Similarly, ingestion of  $\alpha$ -LA can lower lipid triacylglycerol clearance and triacylglycerol secretion downregulation in obese rats (Butler et al., 2009; Kumar et al., 2006; Seo et al., 2012). In aquaculture, lipid is an important energy source for aquatic animals due to its high energy density (Pelt et al., 1997). However, a high lipid content in diets can cause oxidative stress in aquatic animals and lead to lipid peroxidation (Xu et al., 2017; Zy et al., 2008). Considering the role of  $\alpha$ -LA in antioxidation and lipid metabolism regulation in other animals, there is a need to further explore its physiological role in crustacean.

The Chinese mitten crab *Eriocheir sinensis* is an important species in aquaculture and reached 796 622 metric tons in 2014 (Leipnik et al., 2014) with a value of US\$5 547 671 000 (FAO, 2016). In this study, we used *E. sinensis* as a representative species of crabs and aimed to understand the dose-dependent effect of  $\alpha$ -LA in crab fed different lipid percentage from the perspectives of growth performance, body composition, antioxidation and lipid metabolism in *E. sinensis*. The results of this study may provide insights into our understanding of the response of other crustacean species to dietary  $\alpha$ -LA.

## 2. Materials and methods

### 2.1. Experimental diets

Six isonitrogenous practical diets (approximately 35% crude protein) were formulated with different lipid percentage (7% and 13%) and concentration of  $\alpha$ -LA (0, 700 and 1400 mg/kg diet). Fish oil, soybean oil, lecithin and cholesterol were the lipid source. Fish meal, soybean meal and cottonseed meal were the protein source.

Lysine and methionine were added to adjust the balance of amino acids in the diets. Butylated hydroxytoluene (BHT) was added at a concentration of 0.05‰ as the antioxidant to avoid lipid oxidation in the diets. Raw materials were ground and sieved through an 80- $\mu$ m mesh. All dry ingredients were finely ground and mixed thoroughly before adding oil. The mixture was dissolved by adding deionized water (100 mL/kg diet) and then wet-extruded into 2.5-mm-diameter pellets using a double helix plodder (F-26, SCUT industrial factory, Guangdong, China). The scattered pellets were dried by blowing air at room temperature until reaching <10% moisture. Pellets were sieved to various sizes by 12, 10 and 6 mesh sieves and stored at -20 °C until use. Ingredient and proximate composition of the six experimental diets are given in Table 1.

## 2.2. *Experimental crab and management procedure*

Juvenile *E. sinensis* were obtained from a local farm, Shanghai, China. After 5-day acclimation in tanks (300 L) in the Biological Station of East China Normal University, 900 healthy crabs ( $2.90 \pm 0.09$  g) were randomly assigned to 30 tanks (250 L). There were six treatments, each with five replicates, and each replicate with 30 crabs. Six groups of corrugated plastic pipes (12 cm long and 25 mm diameter, six pipes in each group) and six arched tiles were placed in each tank as shelters to reduce attacking behavior. During the experiment, crabs were hand-fed twice daily at 0900 and 1700 hours with the daily ration of 4% body mass for eight weeks. Thirty percent of the daily ration was fed in the morning and 70% in the evening. Two hours after feeding, uneaten diet was removed by siphon. The daily water exchange rate was 50% of the tank volume. The incoming fresh water was aerated thoroughly before entering the water recirculation system. Dead crabs were removed and their weight was recorded. During the whole trial, water temperature ranged from 23.0 to 25.0 °C and pH ranged from 7.6 to 8.4. Dissolved oxygen level was >7.0 mg/L and ammonia-N was <0.05 mg/L.

## 2.3. *Sample collection*

At the end of the eight-week trial, all crabs were fasted for 24 hours and then six crabs at the stage of intermoult from each tank were randomly collected and kept at -20 °C for whole-body composition analysis. Subsequently, crabs were anesthetized in slurry ice and were dissected to obtain the hepatopancreas. All hepatopancreases were stored at -80 °C for further biochemical and molecular analyses. This research was approved by the Committee on the Ethics of Animal Experiments of East China Normal University.

#### 2.4. Growth performance, survival and hepatosomatic index

At the end of the feeding trial, all crabs were counted and weighed. Each hepatopancreas was also weighed to determine the hepatosomatic index (HSI). Weight gain (WG), specific growth rate (SGR), survival and HSI were evaluated using the following formulae:

$$\text{Weight gain (WG, \%)} = [\text{final weight} - \text{initial weight}] / \text{initial weight} \times 100$$

$$\text{Specific growth rate (SGR, \% day}^{-1}\text{)} = [\ln(\text{final weight}) - \ln(\text{initial weight})] \times 100 / \text{days}$$

$$\text{Survival (\%)} = (\text{final crab number} / \text{initial crab number}) \times 100$$

$$\text{Hepatosomatic index (HSI, \%)} = (\text{wet hepatopancreatic weight} / \text{wet body weight}) \times 100$$

#### 2.5. Whole-body composition analysis

The body composition of all crabs and the proximate composition of all diets were analyzed following the standard methods (AOAC, 1995). The moisture of whole-body samples and diets was analyzed by drying to a constant weight at 105 °C. The crude protein content was measured by the Kjeldahl method (8200, Kjeltex, Foss, Sweden). The total lipid contents of whole body and hepatopancreas were extracted with a chloroform /methanol mixture and a 0.37 mol/L KCl solution following the method of Bligh and Dyer and quantified by gravimetry using the vacuum drying oven (DZF-6050, Jinghong, Ltd, Shanghai, China) (Folch et al., 1951). Samples were carbonized completely on a heating plate (TR-30A, SuDa, China) and then incinerated in a muffle furnace (PCD-E3000 Serials, Peaks, Japan) at 550 °C for six hours to measure the ash content.



## 2.6. Hepatopancreas biochemical analysis

Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC) and total protein were measured by using the iodine starch colorimetric method with the commercial assay kits (Cat. No. A003-1, A001-1, A005, A015 and A045-2, Jiancheng, Bioengineering Institute, Nanjing, China). Hepatopancreases were weighed and homogenized in 10 volumes (v/w) of the pre-chilled saline solution. The homogenates were centrifuged at 1500 g (5415R, Eppendorf, Germany) for 30 min and the supernatants were collected. The supernatants of hepatopancreas homogenates were diluted with a 0.85% saline solution according to the procedure developed in respective preliminary experiments before the formal test. The measurements of MDA, SOD, GSH-Px, T-AOC and total protein were based on the methods of Buege 1978, Nebot 1993, Reiners 1991, Benzie & Strain 1996 and Bradford 1976, respectively.

## 2.7. Gene expressions of *se\_TGL*, *se\_IL* and *se\_CPT-1* in hepatopancreas

Total RNA in the six replicaed hepatopancreas was extracted with the Trizol reagent (RN0101, Aidlab, China). Isolated RNA quantity and quality were estimated using a Nano Drop 2000 spectrophotometer (Thermo, Wilmington, USA). For each sample, 1  $\mu$ g of total RNA was reversely transcribed using the PrimeScript<sup>TM</sup> RT Master Mix (RR047A, Takara, Japan) at 42 °C for 2 min to remove genomic DNA and 37 °C for 15 min plus 85 °C for 5 sec to complete reverse transcription. cDNA was unified according to the measured concentration and the standard curve before the real-time quantitative PCR (RT-PCR). Each gene of one treatment was run in six copies with  $\beta$ -actin (GenBank accession no. KM244725.1) as the internal control. Primers of *se\_TGL*, *se\_IL* and *se\_CPT-1* were designed and validated by Primer Premier 6.0 according to the sequence information in our preliminary trials.

The RT-PCR was performed in a final volume of 20  $\mu$ L containing 10  $\mu$ L of 2 $\times$  Ultra SYBR mixture (CW0957, KangWei, China), 0.5  $\mu$ L of 10 mM gene-specific forward and reverse primers, 2.5  $\mu$ L of diluted first-standard cDNA template and 6.5

$\mu\text{L}$  of  $\text{H}_2\text{O}$  with the following cycling conditions: initial denaturation at  $95\text{ }^\circ\text{C}$  for 30 sec, followed by 40 cycles of  $94\text{ }^\circ\text{C}$  for 15 sec,  $58\text{ }^\circ\text{C}$  for 20 sec,  $72\text{ }^\circ\text{C}$  for 20 sec and a  $0.5\text{ }^\circ\text{C}$  per 5 sec increment from  $60\text{ }^\circ\text{C}$  to  $95\text{ }^\circ\text{C}$ . The RT-PCR was conducted in the CFX96 Real-Time PCR system (Bio-rad, Richmond, CA). The cycle time (Ct) values of different treatments were compared to their corresponding internal control and then converted to fold change values by comparing to the control group (7% lipid diet without ALA). CFX Manager™ Software (version 1.0) and Relative Expression Software Tool 384 v. 1 (REST) (Pfaffl, 2002) were used for data visualization and relative quantification analysis.

### 2.8. Statistical analyses

Statistical analysis was carried out with the SPSS statistics 20 (IBM, Armonk, NY, USA). All data are presented as mean  $\pm$  standard errors (SE). The results were subjected to check for normality and homogeneity of variances by Levene's equal variance test. Two-way analysis of variance (ANOVA) was used to determine the significance of main effects of the diet lipid percentage,  $\alpha$ -LA concentration and their interaction (Table 2, 3 and 4). If a significant interaction was detected between the main effects, the post hoc was used to examine the dependent relationship between six treatments. The pair-wised post-hoc comparison was made by Duncan's multiple range test to determine significant difference in *se\_TGL*, *se\_IL* and *se\_CPT-1* mRNA expressions (Fig. 1) in the hepatopancreas. Differences were regarded as statistically significant at  $P < 0.05$  and extremely significant at  $P < 0.01$ .

## 3. Results

### 3.1. Growth performance and survival

No significant difference was found in crab survival among all treatments. The weight gain and SGR increased with the increase of  $\alpha$ -LA addition regardless of the dietary lipid percentage ( $P < 0.05$ ). Crabs fed 13% lipid showed significantly higher HSI than those fed 7% lipid ( $P < 0.01$ ). The  $\alpha$ -LA had no significant influence on HSI at different dietary lipid percentage (Table 2).

### 3.2. Whole-body proximate composition

There was no significant difference in moisture, crude protein and ash among all treatments. The crude lipid of crabs fed 13% lipid was significantly higher than those fed 7% lipid ( $P < 0.01$ ). Meanwhile,  $\alpha$ -LA significantly increased the crude lipid in crabs regardless of dietary lipid percentage ( $P < 0.05$ ) (Table 3).

### 3.3. Hepatopancreatic lipid content

The lipid content in the hepatopancreas was significantly higher in crabs fed 13% lipid than those fed 7% lipid ( $P < 0.01$ ). The lipid in hepatopancreas also significantly increased with the increase of  $\alpha$ -LA concentration in the diets ( $P < 0.01$ ) (Fig. 1-A).

### 3.4. mRNA expressions of lipid decomposition related genes

The mRNA expressions of *se\_TGL* in the hepatopancreas showed an increasing tendency with the increase of  $\alpha$ -LA addition in crabs fed 7% lipid although there was no significant difference. Similarly, there was also no significant difference in *se\_TGL* mRNA expression among the three  $\alpha$ -LA groups in the crabs fed 13% lipid (Fig.1-B). The  $\alpha$ -LA induced significant upregulation of *se\_IL* mRNA expression in the crabs fed 7% lipid ( $P < 0.05$ ) and induced a decreasing trend of expression with increasing  $\alpha$ -LA in the crabs fed 13% lipid (Fig.1-C). No significant difference was found in *se\_CPT-1* mRNA expression among all the treatments (Fig.1-D).

### 3.5. Antioxidative parameters in hepatopancreas

The dietary lipid percentage significantly influenced the activities of SOD ( $P < 0.01$ ) and GHS-Px ( $P < 0.05$ ) in the hepatopancreas. The MDA content was significantly influenced by diet lipid content ( $P < 0.01$ ) and  $\alpha$ -LA addition ( $P < 0.05$ ). T-AOC activities showed a similar pattern to the MDA content in the hepatopancreas although there was no significant difference in T-AOC activities among the six dietary treatments. Crabs fed the diet with 1400 mg  $\alpha$ -LA /kg showed significantly higher activities of SOD and GHS-Px than those fed the other two concentrations of the  $\alpha$ -

LA diets containing 7% crude lipid ( $P < 0.05$ ). In the 13% lipid diet, the GSH-Px activity was lowered with the increase of  $\alpha$ -LA supplementation. No significant difference was found in the SOD activity in the crabs fed 13% lipid (Table 4).

#### 4. Discussion

To the best of our knowledge, this study was the first time to examine the role of  $\alpha$ -LA in the diet of crabs. As an essential coenzyme of the mitochondrial respiratory enzymes in TAC,  $\alpha$ -LA plays a vital function in metabolism regulation and energy transformation (Trattner et al., 2007). In the present study, the administration of  $\alpha$ -LA in the diet with the doses of 700 and 1400 mg/kg significantly improved growth performance of *E. sinensis* compared to the control diet regardless of dietary lipid percentage (Table 2), which is consistent with other studies in abalone *Haliotis discus hannai* (Zhang et al., 2010), Arbor Acres broilers (Guo et al., 2014; Yasin et al., 2012), Hainan black goats (Wang et al., 2015) and hybrid tilapia *Oreochromis niloticus*  $\times$  *Oreochromis aureus* (Xiong et al., 2012) with appropriate  $\alpha$ -LA supplementation. In contrast, the higher doses of  $\alpha$ -LA in the diet can suppress growth performance compared to the control group (Kütter et al., 2012; Zhang et al., 2010). The  $\alpha$ -LA can enhance energy expenditure by regulating the activity of hypothalamic AMPK to induce negative impact on growth performance when  $\alpha$ -LA in the diet is overdosed (Chen et al., 2012; Kim et al., 2004). Clearly, dietary  $\alpha$ -LA can enhance growth performance of animals only within a certain range. However, further research is needed to explore the optimal concentration of  $\alpha$ -LA in the *E. sinensis* diet.

The  $\alpha$ -LA has the ability to prevent dyslipidemia induced by a high-fat diet and reduce the atherosclerosis risk factor in rats by changing the lipid profile (Seo et al., 2012; Xu et al., 2012; Yang et al., 2008). Similarly,  $\alpha$ -LA exerts an anti-obesity effect by suppressing the hypothalamic AMPK activity in rats (Kim et al., 2004). The  $\alpha$ -LA can also induce adipose ATGL protein expression and suppress FAS protein production to decrease lipid content in HepG2 cells and C<sub>2</sub>C<sub>12</sub> myotubes through activation of the AMPK signaling pathway (Chen et al., 2012; Kuo et al., 2012). Surprisingly, supplementation of  $\alpha$ -LA in the crab diet significantly increased lipid

accumulation in the whole body and hepatopancreas of *E. sinensis* in our study (Table 3 and Fig. 1-A). As triacylglycerol lipase (TGL) is an important digestive enzyme and plays a paramount role to break down triacylglycerides (TAGs) from dietary lipids into free fatty acid and glycerol (Cherif et al., 2007; Miled et al., 2000). The mRNA expressions of TGL in hepatopancreas increased with the increase of dietary  $\alpha$ -LA addition in the 7% lipid diet (Fig. 1-B). It is apparent that  $\alpha$ -LA has the ability to accelerate the digestive efficiency in the hepatopancreas of *E. sinensis* fed 7% lipid. Besides,  $\alpha$ -LA supplementation also increased the mRNA expression of intracellular lipase (IL), which has the ability to hydrolysize lipid droplets stored in the hepatopancreas R-cells of crustaceans (Riverap rez C et al., 2011). These results suggest that  $\alpha$ -LA can induce more active metabolism for TAGs digestion and utilization in the hepatopancreas of the crabs fed 7% lipid. Similarly, total lipid in the rat brain was higher in the non-diabetic group with  $\alpha$ -LA injection than those in the control group (Ozkan et al., 2005). In crabs fed 13% lipid,  $\alpha$ -LA supplementation suppressed the mRNA expressions of IL in the hepatopancreas (Fig. 1-C). Therefore,  $\alpha$ -LA may induce lipid accumulation in the *E. sinensis* fed either 7% or 13% lipid. Although the result in the present study differs from others, it may provide a hint to explain the improved reproduction in *E. sinensis* due to the high transfer efficiency of lipid from the hepatopancreas to ovaries during maturation (Teshima et al., 1988; Wen et al., 2001).

Excessive lipid intake and accumulation can increase the risk of oxidative stress in an organism (Keaney et al., 2003). The hepatopancreas in crustacean is a vital organ involved in lipid metabolism in the intestinal, hepatic, pancreatic and adipose tissues (Vogt, 1996). The hepatopancreas is, to a certain degree, analogous to the insect fat body and spider midgut diverticula (Arrese and Soulages, 2010; Laino et al., 2009). In our study, the hepatopancreas in the crabs fed 700 mg/kg  $\alpha$ -LA contained significantly higher MDA than the crabs in the control group fed 7% lipid (Table 4). When  $\alpha$ -LA supplementation increased to 1400 mg/kg diet, the MDA content recovered to the level of the control crab, accompanied by a significant increase of SOD and GSH-Px activities in the hepatopancreas. The increased SOD and GSH-Px

antioxidative enzymes could scavenge excess ROS (Giulio et al., 1995; Halliwell, 1994). Abalone *Haliotis discus hannai* fed an  $\alpha$ -LA-supplemented diet can increase the antioxidant capacity of SOD and GSH-Px activities at the dose of 800 mg/kg diet (Zhang et al., 2010). Similarly, ROS in the brain was significantly reduced when pepper cory *Corydoras paleatus* were fed a diet with  $\alpha$ -LA supplementation (Monserrat et al., 2008). Therefore, we believe that  $\alpha$ -LA in the diet with 1400 mg/kg can improve oxidative stress caused by lipid accumulation in the hepatopancreas of *E. sinensis* fed 7% lipid.

Crabs fed 13% lipid had a significantly higher HSI than those fed 7% lipid. Lipid peroxidation was presented due to excessive ROS in cells by oxidative damage caused by high lipid intake in our study. The MDA contents were slightly increased and the activities of GSH-Px were significantly decreased in the crab hepatopancreas due to lipid accumulation in the 13% diet lipid feeding regime. Meanwhile, T-AOC activities also increased, reflecting the enhanced ability to scavenge ROS (Lewis et al., 1995). The  $\alpha$ -LA as one of the non-enzymatic antioxidant compounds can scavenge a broader range of ROS both in water soluble and lipid soluble environments (Trottner et al., 2007; Yoshikawa et al., 1996) and its reduced form in dihydrolipoic acid (DHLLA) can recover the functions of multiple non-enzymatic antioxidants such as vitamin C, vitamin E and glutathione from their radical forms (Packer et al., 1995). Therefore, it seems that  $\alpha$ -LA plays a positive role in the non-enzyme system for antioxidant defense to protect the hepatopancreas from oxidative damage due to lipid accumulation. However, the supplementation of  $\alpha$ -LA in the diet may not be enough to relieve the damage caused by lipid peroxidation. Further study is needed to identify the optimal concentration of  $\alpha$ -LA supplementation in the diet of Chinese mitten crab.

## 5. Conclusion

The  $\alpha$ -LA supplementation in the diet significantly improved growth performance and accelerated lipid accumulation in the hepatopancreas of *E. sinensis* regardless of dietary lipid percentage. The  $\alpha$ -LA at the dose of 1400 mg/kg effectively reduced oxidative damage caused by excessive lipid deposition in the hepatopancreas

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of *E. sinensis* fed 7% lipid. In crabs fed 13% lipid, although  $\alpha$ -LA in our experimental doses could not relieve the suppression of antioxidative enzyme activities, it increased the total antioxidant capacity in the hepatopancreas of *E. sinensis*. However, further research is needed to determine the optimal range of  $\alpha$ -LA in the *E. sinensis* diet.

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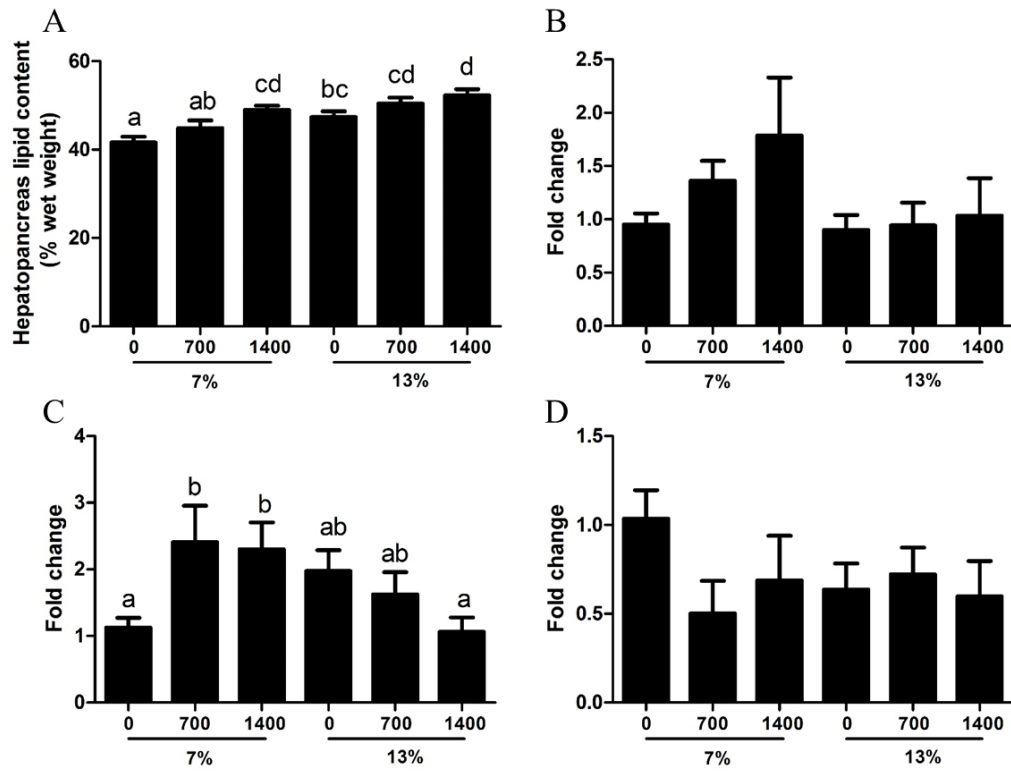


Figure 1

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**Figure Legends****Figure 1**

(A) Lipid content (% wet weight), (B) TGL, (C) IL and (D) CPT-1 mRNA expressions in the hepatopancreas of *E. sinensis* fed two lipid levels and three  $\alpha$ -LA additions.

Values (mean  $\pm$  SE) in bars that show the same letter or nothing are not significantly different ( $P > 0.05$ ) among treatments. The numbers of 0, 700 and 1400 represent 0, 700 and 1400 mg/kg diet  $\alpha$ -LA supplementation, respectively. 7% and 13% represent 7% and 13% lipid content in diet. Values of hepatopancreas lipid are the mean of 16 replicates and values of mRNA expressions are the mean of eight replicates.

**Table 1**

Ingredient formulation (g/kg dry basis) and proximate composition (%) of the six experimental diets fed to *Eriocheir sinensis*

	7% lipid			13% lipid		
	0	700	1400	0	700	1400
Fish meal	250	250	250	250	250	250
Soybean meal	190	190	190	190	190	190
Cottonseed meal	190	190	190	190	190	190
Corn starch	150	150	150	150	150	150
Fish oil: Soybean oil (1:1)	35	35	35	95	95	95
Lecithin	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5
Lysine <sup>a</sup>	4	4	4	4	4	4
Methionine <sup>a</sup>	6	6	6	6	6	6
Vitamin premix <sup>b</sup>	30	30	30	30	30	30
Mineral premix <sup>c</sup>	20	20	20	20	20	20
Choline chloride	5	5	5	5	5	5
Carboxymethyl cellulose	20	20	20	20	20	20
Cellulose	90	90	90	30	30	30
Butylated hydroxytoluene	0.05	0.05	0.05	0.05	0.05	0.05
$\alpha$ -lipoic acid (mg/kg diet) <sup>d</sup>	0	700	1400	0	700	1400
Total	1000	1000	1000	1000	1000	1000
<i>Analysed proximate composition</i>						
Moisture	9.69	10.14	9.68	9.71	9.80	9.85
Crude protein	34.64	35.15	34.58	34.74	34.68	35.02
Crude lipid	7.53	7.59	7.56	13.55	13.31	13.38
Ash	9.00	9.00	9.12	8.85	9.17	9.02

<sup>a</sup> Hainachuan pharmaceutical, Ltd., Guangdong, China

<sup>b</sup> Vitamin premix (per 100 g premix): retinol acetate, 0.043 g; thiamin hydrochloride, 0.15 g; riboflavin, 0.0625 g; Ca pantothenate, 0.3 g; niacin, 0.3 g; pyridoxine hydrochloride, 0.225 g; para-aminobenzoic acid, 0.1 g; ascorbic acid, 0.5 g; biotin, 0.005 g; folic acid, 0.025 g; cholecalciferol, 0.0075 g;  $\alpha$ -tocopherol acetate, 0.5 g; menadione, 0.05 g; inositol, 1 g. All ingredients are filled with  $\alpha$ -cellulose to 100 g.

<sup>c</sup> Mineral premix (per 100 g premix):  $\text{KH}_2\text{PO}_4$ , 21.5 g;  $\text{NaH}_2\text{PO}_4$ , 10.0 g;  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 26.5 g;  $\text{CaCO}_3$ , 10.5 g; KCl, 2.8 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 10.0 g;  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.024 g;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.476 g;  $\text{MnSO}_4 \cdot 6\text{H}_2\text{O}$ , 0.143 g; KI, 0.023 g;  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.015 g;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.14 g; Calcium lactate, 16.50 g; Fe- citrate, 1 g. All ingredients are diluted with  $\alpha$ -cellulose to 100 g.

<sup>d</sup> Sangon Biotech, Ltd., Shanghai, China.



**Table 2**

Growth performance and survival of *Eriocheir sinensis* fed differing in lipid levels and  $\alpha$ -lipoic acid contents (mean  $\pm$  SE).

Diets				
Lipid (%) - $\alpha$ -lipoic acid (mg/kg diet)	WG (%)	SGR (%)	HSI (%)	Survival (%)
7-0	92.28 $\pm$ 10.59 <sup>a</sup>	1.17 $\pm$ 0.10 <sup>a</sup>	7.49 $\pm$ 1.00 <sup>a</sup>	93.33 $\pm$ 6.24
7-700	102.58 $\pm$ 14.82 <sup>ab</sup>	1.26 $\pm$ 0.13 <sup>ab</sup>	7.58 $\pm$ 1.00 <sup>a</sup>	89.33 $\pm$ 4.94
7-1400	112.90 $\pm$ 7.00 <sup>b</sup>	1.35 $\pm$ 0.06 <sup>b</sup>	7.50 $\pm$ 0.64 <sup>a</sup>	90.00 $\pm$ 4.71
13-0	90.91 $\pm$ 7.03 <sup>a</sup>	1.15 $\pm$ 0.07 <sup>a</sup>	7.98 $\pm$ 1.11 <sup>ab</sup>	94.00 $\pm$ 3.65
13-700	92.58 $\pm$ 17.66 <sup>a</sup>	1.16 $\pm$ 0.17 <sup>a</sup>	8.00 $\pm$ 1.02 <sup>ab</sup>	94.67 $\pm$ 3.80
13-1400	103.59 $\pm$ 15.78 <sup>ab</sup>	1.27 $\pm$ 0.14 <sup>ab</sup>	8.23 $\pm$ 0.84 <sup>b</sup>	90.83 $\pm$ 4.19
ANOVA ( <i>P</i> )				
Lipid	0.166	0.164	0.002	0.205
$\alpha$ -lipoic acid	0.031	0.034	0.821	0.340
Lipid $\times$ $\alpha$ -lipoic acid	0.732	0.720	0.743	0.469

WG, weight gain; SGR, specific growth rate; HSI, hepatosomatic index.

<sup>a, b</sup> Values within a column without a common superscript letter are different (b indicated the higher value). *P* < 0.05 means significant difference; *P* < 0.01 means extreme significant difference. Values of WG and SGR are mean of 5. Values of HSI are mean of 20.

**Table 3**

Proximate composition of *Eriocheir sinensis* (% wet weight) fed diets differing in lipid levels and  $\alpha$ -lipoic acid contents (mean  $\pm$  SE).

Diets	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Lipid (%) - $\alpha$ -lipoic acid (mg/kg diet)				
7-0	63.86 $\pm$ 1.28	13.48 $\pm$ 0.39	4.93 $\pm$ 0.73 <sup>a</sup>	13.14 $\pm$ 0.75
7-700	63.28 $\pm$ 1.72	13.59 $\pm$ 0.76	5.50 $\pm$ 0.18 <sup>ab</sup>	13.18 $\pm$ 0.50
7-1400	63.56 $\pm$ 3.91	13.70 $\pm$ 1.32	5.88 $\pm$ 0.45 <sup>bc</sup>	13.22 $\pm$ 1.04
13-0	64.04 $\pm$ 1.22	13.07 $\pm$ 0.34	6.18 $\pm$ 0.27 <sup>bc</sup>	13.23 $\pm$ 0.74
13-700	62.05 $\pm$ 2.29	13.80 $\pm$ 0.48	6.46 $\pm$ 0.65 <sup>c</sup>	13.58 $\pm$ 0.97
13-1400	61.85 $\pm$ 1.15	13.65 $\pm$ 0.76	6.57 $\pm$ 0.20 <sup>c</sup>	13.79 $\pm$ 0.35
ANOVA ( <i>P</i> )				
Lipid	0.311	0.774	0.000	0.275
$\alpha$ -lipoic acid	0.417	0.472	0.029	0.704
Lipid $\times$ $\alpha$ -lipoic acid	0.665	0.716	0.494	0.819

<sup>a, b, c, d</sup> Values within a column without a common superscript letter are different (d indicated the highest value). *P* < 0.05 means significant difference; *P* < 0.01 means extreme significant difference.

Values of moisture, crude protein, crude lipid and ash are mean of 5.

**Table 4**

Hepatopancreas contents of protein, MDA and activities of SOD, GSH-Px and T-AOC of *Eriocheir sinensis* fed diets differing in lipid levels and  $\alpha$ -lipoic acid contents (mean  $\pm$  SE).

Diets	Protein (mg/mL)	MDA (nmol/mgprot)	SOD (U/mgprot)	GSH-Px (U/mgprot)	T-AOC (U/mgprot)
Lipid (%) - $\alpha$ -lipoic acid (mg/kg diet)					
7-0	485.36 $\pm$ 10.86	0.10 $\pm$ 0.01 <sup>a</sup>	17.61 $\pm$ 2.14 <sup>a</sup>	61.98 $\pm$ 7.76 <sup>abc</sup>	0.77 $\pm$ 0.09
7-700	488.91 $\pm$ 10.13	0.15 $\pm$ 0.01 <sup>cd</sup>	17.65 $\pm$ 0.35 <sup>a</sup>	58.49 $\pm$ 15.61 <sup>abc</sup>	0.89 $\pm$ 0.05
7-1400	472.94 $\pm$ 9.47	0.11 $\pm$ 0.01 <sup>ab</sup>	22.24 $\pm$ 0.74 <sup>b</sup>	81.90 $\pm$ 12.86 <sup>c</sup>	0.79 $\pm$ 0.05
13-0	480.55 $\pm$ 52.68	0.13 $\pm$ 0.01 <sup>bc</sup>	25.42 $\pm$ 0.99 <sup>b</sup>	70.33 $\pm$ 7.42 <sup>bc</sup>	0.79 $\pm$ 0.03
13-700	492.62 $\pm$ 81.89	0.14 $\pm$ 0.01 <sup>c</sup>	25.49 $\pm$ 0.72 <sup>b</sup>	52.73 $\pm$ 7.74 <sup>ab</sup>	0.95 $\pm$ 0.11
13-1400	487.87 $\pm$ 49.24	0.16 $\pm$ 0.01 <sup>d</sup>	23.80 $\pm$ 0.93 <sup>b</sup>	36.07 $\pm$ 8.75 <sup>a</sup>	1.06 $\pm$ 0.13
ANOVA ( <i>P</i> )					
Lipid	0.686	0.003	0.000	0.049	0.088
$\alpha$ -lipoic acid	0.741	0.012	0.321	0.470	0.172
Lipid $\times$ $\alpha$ -lipoic acid	0.777	0.001	0.008	0.010	0.282

MDA, malondialdehyde. SOD, superoxide dismutase. GSH-Px, glutathione peroxidase. T-AOC, total antioxidant capacity.

<sup>a, b, c, d</sup> Values within a column without a common superscript letter are different (d indicated the highest value). *P* < 0.05 means significant difference; *P* < 0.01 means extreme significant difference.

Values of hepatopancreas protein, MDA, SOD, GSH-Px and T-AOC are the mean of eight replicates.

**Table 5**

Primer pair sequences and product size of the genes used for real-time PCR (qPCR)

Gene	Position	5'-3' primer sequence	Length	Tm	Product size (bp)
TGL	Forward	CTACAACTTCCTCTTCCTGAT	21	53.7	118
	Reverse	GTAGTGGTTGACGGTGTG	18	54.9	
IL	Forward	CGATCCTACGAGTTCTTCA	19	53	125
	Reverse	GCACTTGGTGTTGTCATC	18	52.6	
CPT-1	Forward	GAATCTGTGACGGAGGAG	18	54.9	97
	Reverse	TGGCTGACGATGGTTATG	18	52.6	
$\beta$ -actin	Forward	ATCACTGCTCTTGCTCCT	18	53.4	145
	Reverse	ACTCGTCGTATTCCTCCTT	19	53.5	

GTL, triacylglycerol lipase. IL, intracellular lipase. CPT-1, carnitine palmitoyl transferase-1.

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**Highlights**

1. Dietary  $\alpha$ -lipoic acid can significantly improve the growth of *E. sinensis*.
2.  $\alpha$ -lipoic acid accelerates crab hepatopancreas lipid accumulation in a dose-dependent manner.
3. Dietary  $\alpha$ -lipoic acid of 1400 mg/kg can reduce oxidative damage by excessive lipid deposition in *E. sinensis*.

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