# Lipometabolic Alteration in Mice Feeding Eatable Tissues of Chinese Mitten Crab

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## Abstract

Objective: Chinese mitten crab is a famous aquatic species in eastern Asian region, but their edible parts, particularly hepatopancreas and gonads, generally contain very high levels of lipids that may have negative effects on human health. This study investigated the effects of different edible parts of Chinese mitten crab on the body weight and lip metabolism for Kunming mice.

Method: The mice were fed with diets containing one part of an Chinese mitten crab or the mixture of parts of an Chinese mitten crab for 4 weeks. There were 9 treatments. The triacylglycerol (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were enzymatically determined using commercial kits (purchased from Nanjing Jiancheng Bioengineering Institute, China). The arteriosclerosis index (AI) was calculated by the equation: AI = (TC - HDL-C)/HDL-C. The levels of fatty acid syntheses (FAS), the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA) and lipoprotein lipase (LPL) were measured using commercially available kits according to the manufacturer's instructions. The significant differences between the groups were further analyzed by Bonferronis's t-test.

Results: Our results showed that the crab hepatopancreas, gonads and the mixed male crab-edible parts increased blood lipids in some experiment group of mice corresponding to a change in the nutrition-related liver enzymes. It shows that addition of the Chinese mitten crab has an adverse effect on the blood lipid levels in mice. The FFH, FFMI and FMMI groups had significantly higher weight than the FN group (P < 0.05). The crab hepatopancreas, crab gonads and the mixed male crab-edible parts cause an increase in the blood lipid levels. The crab mixture significantly affected the AI value of male and female mice (P < 0.01). The level of FMMI group was significantly higher than the FN group (P < 0.05). Other groups showed no significant difference. The level of the FFMI group was significantly lower than the FN group (P < 0.05), and levels in the MMM and MFMI groups were significantly lower than the MN group (P < 0.05).

Conclusion: It clearly showed that long-term feeding with the Chinese mitten crab has an adverse effect on the blood lipid levels in mice. One the one hand, the weight, liver index and fat index of experimental mice were changed than normal mice. On the other hand, the crab diet affects the level of TC, TG, AI and FASN on increasing. It is suggested that the special diet has affected lip metabolic alteration associated with contents of serum lipids and metabolic enzymes. But according to a certain regular feeding, there would be no adverse effect on mice. On the contrary, it may adjust the blood lipid in mice

Keywords: blood lipid, Chinese mitten crab, liver enzyme, mice

## 1. Introduction

Chinese mitten crab has delicious taste with a unique and pleasant aroma. It also has good nutritive value including high levels of fatty acids and amino acids (Guo, Gu, Wang, Zhao, & Zheng, 2014). There are three quality ranks to evaluate the value of a Chinese mitten crab according to Chinese National Standard GB/T 19957-2005, and they are separated mainly based on weight [General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (2005) GB/T 19957-2005 Product of geographical indication-Yangcheng Lake Chinese mitten crab. Standards Press of China, Beijing]. These include special, first, and second classes (Gs, G1, and G2), for which male/female crabs should weigh over 200/150 g, 150/125 g, and 125/100 g, respectively. They differ in nutrition contents and flavors.

Table 1. Proximate composition (% of wet weight) in hepatopancreas, mature gonads and muscle of adult Chinese mitten crab (Wu, 2007)

	Yield (%)	Moisture (%)	Protein (%)	Total lipid (%)	Ash (%)
Male crab muscle	23-28	75-80	17-20	0.8-1.2	1.2-1.5
Male crab hepatopancreas	3.5-6	52-65	9-14	9-16	1.6-2.3
Male crab gonads	2.5-4.5	70-73	16-19	1.5-2	2.4-2.8
Female crab muscle	21-25	72-78	18-20	1.5-2.5	2.1-2.4
Female crab hepatopancreas	4.0-7.5	37-48	10-17	17-36	1.4-2
Female crab gonads	8.0-13.0	40-50	30-35	16-19	2-2.5

In general, Chinese mitten crab contains 18.9% crude protein, and about 80% of the protein is in the crab meat portion. About 90% of the fat is in the viscera (Chen, Zhang, & Shrestha, 2007) (Table 1). The Chinese mitten crab has an elevate highly unsaturated fatty acid (HUFA) levels in their gonad and hepatopancreas (Guo, Gu, Wang, Zhao, & Zheng, 2014; Wu et al., 2007). It has been reported that fatty acid profiles, especially of essential fatty acids (EFAs), are closely related to nutrient quality. The EFAs consist of a-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6). ALA is a precursor of n-3 fatty acids, including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), while LA is a precursor of arachidonic acid (AA, 20:4n-6) (Gil, 2002).

AA and DHA are major components of cell membrane phospholipids and abundant in the central nervous system (He et al., 2014; Singh, 2005). EPA and DHA are important in infant brain growth (Wu et al., 2007) and have cardio-protective (Lee, O'Keefe, Lavie, Marchioli, & Harris, 2008; Ross, Lombardo, & Chicco, 2010) and anti-cancer (Alberts & Greenspan, 1984; Donaldson, 2004) properties. Balancing the nutritive material with risk is important.Crab meat is also an excellent source of minerals, particularly calcium, iron, zinc, potassium and phosphorus (Naczk, Williams, Brennan, Liyanapathirana, & Shahidi, 2004).

One study showed that the feeding gonad and hepatopancreas of Chinese mitten crab enriches fat in mice quickly with normal blood lipids (Su, Li, Ouyang, & Liu, 1995). However, high levels of fat intake may promote pathogenesis of many diseases including Crohn's disease (Calder, 2006) and inflammatory diseases (Gil, 2002). This reports suggests that eating a proper amount of gonad and hepatopancreas of Chinese mitten crab can play an important role in nutrition and biological activities.

The aim of this study was to measure the effect of eating crab gonad and hepatopancreas on blood lipid levels. We will study the weight-gaining effect of Chinese mitten crab consumption and thus determine whether the harm of crab-eating outweighs its merits. The study was 4 weeks and we use serum biochemical indices in mice.

## 2. Materials and Methods

These studies were approved by the university committee for animal experiments.All the experiments followed Chinese legislations on the use and care of laboratory animals.

## 2.1 Animals

Healthy Kunming specie mice, weighing 18-22 g, were purchased from shanghai Slac Laboratory Animal (SCXK2012-0002). The animals were maintained under standard conditions (12 h day/night cycle, 22±2 °C, 50%-60% humidity) with free access to food and tap water. Mice body weights were recorded weekly. Animals were allowed to acclimate to the environment for at least 1 week before use in the described experiments. Then

the female mice were randomly divided into nine groups (n = 10), each group includes 10 mice; Group 1: The normal control group (FN) was fed a normal diet (composed of wheat (30%), ginglly oil cake (25%), black gram husk (29%), soybean meal (15%) and mineral mixture (10%)). Group 2: The FFG group was fed female crab gonads (showing in Table 2, following same) with a normal diet. Group 3: The FMG group was fed male crab gonads with a normal diet. Group 4: The FFM group was fed female crab muscle with a normal diet. Group 5: The FMM group was fed male crab muscle with a normal diet. Group 6: The FFH group was fed female crab hepato-pancreas with a normal diet. Group 7: The FMH group was fed male crab hepatopancreas with a normal diet. Group 8: The FFMI group was fed with a mixture of edible parts from female crab with the normal diet. Group 9: The FMMI group was fed a mixture of edible parts from female crab with a normal diet. The male mice were used and they were under the same treatment. The experimental diet was lasted for 4 weeks.

Groups	Normal diet (%)	Male crab hepatopancreas (%)	Male crab muscle (%)	Male crab gonads (%)	Female crab hepatopancreas (%)	Female crab muscle (%)	Female crab gonads (%)
MMMI FMMI	95.18	0.73	3.65	0.44	0	0	0
FMH MMH	99.27	0.73	0	0	0	0	0
FMM MMM	96.35	0	3.65	0	0	0	0
FMG MMG	99.56	0	0	0.44	0	0	0
MN	100	0	0	0	0	0	0
MFMI FFMI	95.82	0	0	0	0.63	2.4	1.15
FFH FMH	99.37	0	0	0	0.63	0	0
FFM MFM	97.6	0	0	0	0	2.4	0
FMG FMG	98.85	0	0	0	0	0	1.15
FN	100	0	0	0	0	0	0

Table 2. Each group mice feed formulation

Then the mice were sacrificed, and the liver were excised, weighed, and then homogenized for enzymatic analysis.

## 2.2 Estimation of Plasma Lipid Profile

At the end of the experiment, animals were fasted overnight (14 h) and euthanized under diethyl ether anesthesia in the morning by withdrawing blood from the abdominal vena using a vacuum tube. The blood was clotted and plasma was harvested by centrifugation at 4 °C (1800 ×g, 10 min). The triacylglycerol (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were enzymatically determined using commercial kits (purchased from Nanjing Jiancheng Bioengineering Institute, China) with Beckman coulter chemistry analyzer AU5800 Series. The arteriosclerosis index (AI) was calculated by the equation: AI = (TC - HDL-C)/HDL-C.

## 2.3 Enzymatic

The livers were quickly removed and homogenized in 50mM Tris-HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged at 4  $^{\circ}$ C (2400 ×g, 15 min). The levels of FAS, HMG-CoA and LPL were measured using commercially available kits according to the manufacturer's instructions.

## 2.4 Statistical Analysis

All data were represented as mean $\pm$ SD from 10 samples per group. The mean values were statistically analyzed using one-way analysis of variance (ANOVA). The significant differences between the groups were further analyzed by Bonferronis's t-test. Analyses were performed using the SPSS 19.0 software. *P* values less than 0.05 were considered as significant. Plots were made with package ggplot2 (Wickham, 2009) of R (Team, 2014).

## 3. Results and Discussion

## 3.1 Effect of Chinese Mitten Crab on Mice Weight

Body weight and liver tissue weight for each experimental diet group are shown in Tables 3 and 4. In female mice, Chinese mitten crab consumption led to a higher body weight versus normal control diets. This was true both for FFH, FFMI and FMMI groups—they had significantly higher body weight than the FN group (P < 0.05).

All female mice groups had significantly higher body weight gain than the FN group except the FFG group (P < 0.05). However, there was no significant difference in the liver tissue weights (P > 0.05).

Group	Initial body weight(g)	Final body weight (g)	Body weight gain (g)	Liver tissue weights (g/100 g)
FN	24.38±1.18	39.76±5.34a	15.38±2.34a	4.78±0.50
FFG	24.28±1.82	42.32±3.46ab	18.04±2.13ab	4.70±0.51
FMG	23.70±1.28	43.07±3.11ab	19.23±2.23b	4.79±0.48
FFM	24.72±2.74	43.97±2.44ab	19.25±1.21b	4.55±0.37
FMM	24.34±1.89	43.76±2.19ab	19.42±1.54b	5.03±0.54
FFH	24.66±1.12	45.57±2.59b	20.91±1.86b	4.73±0.62
FMH	23.90±1.55	44.57±2.78ab	20.67±2.13b	4.6±047
FFMI	24.64±0.72	46.00±2.73b	21.36±1.34b	4.72±0.38
FMMI	24.48±1.97	45.76±3.14b	21.28±1.24b	4.91±0.32
MMMI	23.54±1.44	37.27±4.48	13.73±2.38	5.61±0.56b

Table 3. Effect of Chinese mitten crab on growth indicators of female mice

In male mice, Chinese mitten crab consumption had only a minimal effect on weight. Both the final body weight and the body weight gain showed no significant difference between the groups (P > 0.05). Liver tissue weights, however, were significantly higher in the MFH, MFMI and MMMI groups than in the MN group (P < 0.05).

Group	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Liver tissue weights (g/100 g)
MN	23.76±1.09	33.31±4.53	9.56±2.39	4.98±0.28ab
MFG	24.20±0.84	32.06±4.34	7.86±3.46	6.22±0.32a
MMG	24.24±1.18	33.58±2.67	9.34±2.13	5.01±0.57ab
MFM	24.00±0.77	32.79±4.11	8.79±2.61	5.49±0.55a
MMM	23.60±1.05	32.92±3.87	9.32±2.42	5.32±0.43ab
MFH	23.60±1.50	35.69±4.13	12.09±2.18	5.75±0.66ab
MMH	24.11±1.09	34.71±3.71	10.6±2.27	5.20±0.58ab
MFMI	23.80±1.43	37.43±4.05	13.63±2.32	6.20±0.35b
MMMI	23.54±1.44	37.27±4.48	13.73±2.38	5.61±0.56b

Table 4. Effect of Chinese mitten crab on growth indicators of male mice

#### 3.2 Effect of Chinese Mitten Crab on TC, TG, HDL-C, LDL-C, and AI

The levels of TC, TG, HDL-C and LDL-C from treatment plasma were displaying in the part of Tables 5 and 6.

Total cholesterol level in the FFG, FFMI, and FMMI groups were significantly higher than that of the FN group (P < 0.05). Compared to the normal control group, TC levels in the FMG and FFH groups were elevated, and FFM, FMM, and FMH groups were reduced but not significantly (P > 0.05). The triglyceride level in the FFM, FMM, FFH and FMMI groups were significantly higher than that of the normal control group (P < 0.05); other groups were elevated, but these differences were not significant (P > 0.05). Chinese mitten crab does not affect the levels of HDL-C and LDL-C. All groups were normal.

The total cholesterol level in the MFG, MMG, MFH and MMMI groups were significantly higher than that in the normal control group (P < 0.05). Furthermore, MFMI group was markedly increased versus the normal control group (P < 0.01). Except for the MMM group, other groups were higher than the normal control group. However, these differences were not significant (P > 0.05). In terms of the triglyceride level, the MFG and MFMI groups were significantly higher than the normal control group (P < 0.05). The HDL-C and LDL-C level in the MFG, MMG, MFH, and MFMI were significantly higher than the normal control group (P < 0.05). The MFH group was very significant (P < 0.01). However, the MMM and MFM groups were significantly lower than the normal control group (P < 0.05). The LDL-C level in the MFMI group was significantly higher than the normal control group (P < 0.05).

The total cholesterol was inclined to elevate alteration in male or female mice treatment because the total cholesterol of main resource is diet including reach cholesterol (Leontowicz, 2011). The triglyceride level of plasma was raising for hyperlipidemia in few treatment groups from the male or female groups. The alteration of HDL-C and LDL-C level was observed in male treatment group. This is consistent with the general principle (Rony, 2014). The eatable tissue of hepatopancreas or gonads was added to the diet which were can raising the lipometabolite level than muscle including lowly lipids amount in mice treatment groups. The lipometabolete level of male mice than the female mice was easily affected by hepatopancreas or gonads or mixed eatable tissue of Chinese mitten crab.

The AI level in the FFMI and FMMI groups were significantly higher than the normal control group (P < 0.01). Other groups were higher than the normal control group, but these differences were not significant (P > 0.05).

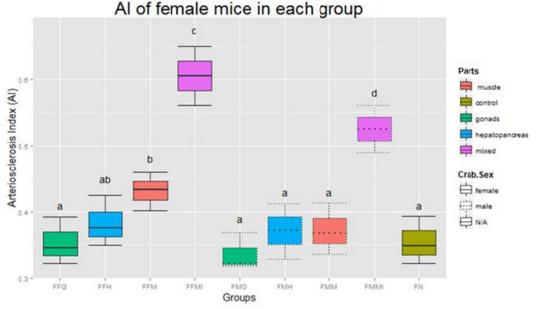


Figure 1. AI of female mice in each group

The AI level (Figure 1) in the MFG, MMM, MMH, MFMI and MMMI groups were significantly greater than that of the normal control group (P < 0.01). Other groups were higher than that of the normal control group, but these differences were not significant (P > 0.05).

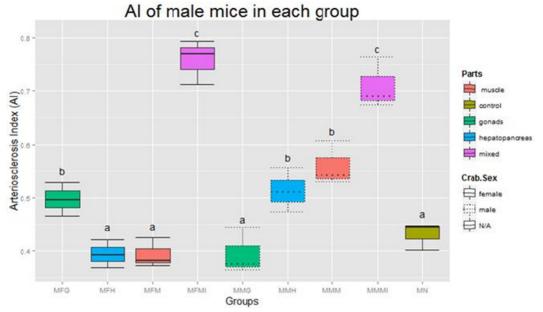


Figure 2. AI of male mice in each group

The AI level (Figure 1) in the MFMI and MMMI groups were significantly greater than that of the MN group by 75.93% and 64.54%, respectively (P < 0.01). The AI level in the MFG, MMM and MMH groups were significantly greater than the normal control group (P < 0.05).

The AI of treatment groups as few eating mixed parts were significantly greater in male mice or female mice. The edible gonads have caused a lowly AI value on treatment groups.

3.3 Effect of Chinese Mitten Crab on FAS, HMG-CoA, and LPL

The levels of FAS, HMG-CoA, and LPL from the liver tissue were displaying as well in the part of Tables 5 and 6.

Group	FAS	HMG-CoA reductase	LPL	TC	TG	HDL-C	LDL-C
oroup	(nmol/g protein)	(ng/g protein)	(U/g protein)	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)
FN	2.93±0.07cd	116.88±6.82abcd	70.15±3.36ce	1.64±0.12abc	0.85±0.10a	1.17±0.12ab	0.34±0.08abc
FFG	2.97±0.22cd	109.08±3.59ab	63.61±7.31abcd	1.99±0.12de	0.85±0.07a	1.33±0.16b	0.42±0.07bc
FMG	2.87±0.15c	113.47±5.40abc	66.71±5.53bcd	1.76±0.20de	0.98±0.11ab	1.28±0.27ab	0.43±0.06bc
FFM	2.64±0.14abc	120.43±2.01cd	67.89±3.59bcd	1.41±0.23a	1.20±0.13b	1.02±0.22ab	$0.31 \pm 0.08 ab$
FMM	2.52±0.14ab	119.95±7.36cd	64.75±4.35abcd	1.48±0.19ab	1.21±0.19b	0.96±0.20a	0.31±0.06ab
FFH	2.81±0.22bc	126.58±5.28d	60.61±5.03ab	1.88±0.16cde	1.07±0.20ab	1.35±0.14b	0.45±0.05c
FMH	2.46±0.21q	118.36±2.56bcd	61.46±2.04abc	1.54±0.14cde	1.01±0.10ab	1.02±0.12ab	0.31±0.06ab
FFMI	3.21±0.25de	107.21±5.35a	56.58±5.18a	2.06±0.11de	1.05±0.09ab	1.10±0.11ab	0.28±0.05a
FMMI	3.44±0.21e	115.34±7.91abc	70.54±2.17d	2.10±0.21e	1.16±0.09b	1.19±0.19ab	0.29±0.07a

Table 5. Effect of Chinese mitten crab on FAS, HMG-CoA, LPL, TC, TG, HDL-C and LDL-C of female mice

The FAS of FMMI group (Table 5) was significantly higher than the FN group (P < 0.05). In the male mice (Table 6), the level of FAS in MFG, MMG, MFH, MFMI and MMMI groups were significantly higher than that of the MN group (P < 0.05). Other groups showed no significant difference. The triglyceride stored in adipose tissue originates either from the esterification of FFAs provided mainly from the diet or from denovo synthesis. The activity of the lipogenic pathway in adipose tissue is highly dependent on nutritional conditions (Rossi, 2010). Fatty acid synthetase (FAS) is the designation given to the enzyme system that catalyzes the synthesis of long-chain fatty acids from a short-chain acyl-CoA primer (Alberts & Greenspan, 1984).

Group	FAS (nmol/g protein)	HMG-CoA reductase (ng/g protein)	LPL (U/g protein)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
MN	2.91±0.09cde	113.51±5.89ab	65.25±8.27ab	1.65±0.22abc	0.83±0.16abc	1.25±0.12b	0.27±0.06a
MFG	3.16±0.38def	118.64±5.64b	60.24±7.21ab	1.95±0.18cd	1.27±0.11e	1.45±0.08c	0.33±0.04ab
MMG	2.76±0.06bcd	108.30±9.05ab	64.87±3.25ab	1.89±0.23bcd	1.02±0.17cde	1.43±0.12c	0.29±0.06ab
MFM	2.38±0.11b	104.99±9.98ab	60.25±2.11ab	1.59±0.13ab	0.73±0.09ab	0.94±0.07a	0.34±0.10ab
MMM	2.36±0.34b	102.73±8.27a	55.98±4.25a	1.34±0.13a	0.62±0.17a	0.88±0.06a	0.34±0.04ab
MFH	2.70±0.28bc	102.10±6.59a	65.26±3.14ab	1.98±0.20cd	0.94±0.17bcd	1.64±0.09d	0.30±0.09ab
MMH	1.92±0.23a	103.17±4.83a	58.77±6.79ab	1.81±0.16bcd	0.70±0.16ab	1.22±0.11b	0.28±0.05ab
MFMI	3.45±0.14f	118.72±5.20b	55.98±4.25a	2.47±0.13e	1.13±0.22de	1.62±0.09d	0.41±0.04b
MMMI	3.29 ±0.12ef	108.17±7.81ab	66.90±2.53b	1.99±0.17d	1.02±0.06cde	1.24±0.11b	0.35±0.09ab

As for HMG-CoA, the level of this reductase in MFG and MFMI were significantly higher than that in the MMM, MFH, MMH groups (P < 0.05). However, both female and male mice belong to treatment groups showed no significant difference compared with the normal group (P > 0.05). People with higher levels of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase expression, as indicated by their serum mevalonate or cholesterol levels, appear to be more resistant to dietary cholesterol (Ness, 2015). Cholesterol biosynthesis localized in the endoplasmic reticulum (ER) starts with the rate limiting enzyme (HMG-CoA) reductase whose activity is strongly controlled by several feed-back mechanisms involving endogenous pathways and exogenous cholesterol intake by nutrition (Reynolds et al., 1984).

The level of LPL in the FFMI group was significantly lower than that in the FN group (P < 0.05); levels in the MMM and MFMI groups was significantly lower than that in the MN group (P < 0.05). The lipoprotein lipase (LPL) plays an important role in lipid metabolism by hydrolyzing triglycerides in chylomicrons and very low density lipoproteins (Goldberg, 1996). An increasing number of studies have suggested an association of LPL gene variants with the risk of cardiovascular and cerebrovascular diseases (Munshi et al., 2012). Several structural mutations in the LPL gene have been documented (Mead, Irvine, & Ramji, 2002). These have been associated with various lipid traits like hypertriglyceridemia and reduced HDL levels (two polymorphisms in the human lipoprotein lipase (LPL) gene).

## 4. Conclusion

The lipids contents are different in the eatable tissues like gonads, muscle, hepatopancreas which were associated with male and female of Chinese mitten crab (Wu, 2007). The Different lipids contents as an eatable tissues maker of diets were fed with 8 experiment groups including of FFG, FMG, FFM, FMM, FFH, FMH, FFMI and FMMI for female mice. The similar experiment design is in male mice. The list of highest to lowest on the lipids content about diets were postfix MI group, postfix G group, postfix H group and postfix M group (data no showing). The special diet containing of eatable tissues of Chinese mitten crab was affected on the lipometabolite showing in the blood biochemistry index and metabolism enzyme of mice.

Chinese mitten crab was including to the diet has an adverse effect on the blood lipid levels in mice. (1) Mice feed the diet containing eatable tissue of Chinese mitten crab which the body weight was more increased. The FFH, FFMI and FMMI groups had significantly higher body weight than the FN group (P < 0.05); (2) the crab-edible parts was included in the special diet had a greater adverse impacts on the male mice. The crab hepatopancreas, crab gonads and the mixed male crab-edible parts caused an increase in the blood lipid levels. (3) The eatable Crab part increased the lipid index in male and female mice. The crab mixture significantly affected the AI value of male and female mice (P < 0.01). (4) The level of FAS in MFG, MMG, MFH, MFMI and MMMI groups were significantly higher than that of the MN group (P < 0.05). The level of FMMI group was significantly higher than the FN group (P < 0.05). Other groups showed no significant difference. (5) The level of of LPL in the FFMI group was significantly lower than the FN group (P < 0.05), and levels of LPL in the MMM and MFMI groups were significantly lower than the MN group (P < 0.05).

Hyperlipidemia is a major cause of build-up in coronary atherosclerosis (Steinberg, 2005). Whether TC or TG levels increased, or both increased, they are referred to as hyperlipidemia. We found that feeding mice with diets rich in lipids resulted in increased TC, TG and LDL cholesterol levels. The results was in accordance with the the report belong Engelking. Fatty acid synthetase (FAS) is the designation given to the enzyme system which catalyzes the synthesis of long-chain fatty acids from a short-chain acyl-CoA primer (Cole & Kramer, 2016;

Watkins, 2013). The concentration of FAS enzymes in liver affects the concentration of TC and TG in plasma (Myant, 1990). Feeding with the Chinese mitten crab may increase the FAS enzyme level to increase TC and TG. Moreover, HDL is directly anti-androgenic and it is believed to remove cholesterol from the developing lesions. LDL is a risk factor and plays a role in several steps of atherosclerosis.

However, to understand the implications of these findings on humans, we need further research to understand other potential health hazards.

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