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Effects of dietary yeast extract supplementation on growth, body composition, non-specific immunity, and antioxidant status of Chinese mitten crab (*Eriocheir sinensis*)

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ABSTRACT

This study was conducted to evaluate the effects of dietary supplementation with yeast extract on growth, body composition, non-specific immunity, and antioxidant status of Chinese mitten crab (*Eriocheir sinensis*). A total of 432 crabs (initial average weight, 4.62 ± 0.11 g) were randomly distributed into four treatment groups with six replicates of 18 crabs. The crabs were fed a basal diet or the same diet supplemented with 2.5, 5, and 10 g/kg yeast extract for 8 weeks. The results showed that dietary yeast extract inclusion enhanced the edible viscera index (linear, $P < 0.001$), edible viscera crude protein (CP) content (linear, $P = 0.025$) and serum phenoloxidase (ProPO) activity (quadratic, $P = 0.023$) at 56 day, increased the total superoxide dismutase (T-SOD) activity at 28 day (quadratic, $P = 0.037$) and catelase (CAT) activity at 56 day (quadratic, $P = 0.034$) of edible viscera, and muscular T-SOD activity (quadratic, $P = 0.020$) at 56 day in Chinese mitten crab. Compared with the control group, the inclusion of 5 g/kg yeast extract in the diet increased the edible viscera index, enhanced the CAT activity of edible viscera at 56 day in Chinese mitten crab ($P < 0.05$). Dietary 10 g/kg yeast extract inclusion enhanced the edible viscera index at 56 day in Chinese mitten crab than that of the control group ($P < 0.05$). These results implied that dietary yeast extract inclusion improved the edible viscera index and crude protein content of edible viscera, enhanced serum immunity, and increased the antioxidant status of edible viscera and muscle in Chinese mitten crab, especially when it is supplemented at 5 g/kg yeast extract in the diet.

1. Introduction

Yeast extract, as a yeast product separated from inner yeast cells, could be a functional source of nutrients [1,2]. Elsa et al. [3] reported that the yeast extract is a natural ingredient composed of a variety of peptides, nucleotides, B-complex vitamins, and minerals, and high-quality protein rich in essential amino acids. Previous studies reported that yeast extract in the diet could possess the immunoregulatory and antimicrobial capabilities in aquatic animals [4–6]. Jin et al. [5] reported that dietary 10 g/kg yeast product supplementation improved the growth performance, enhanced innate immunity, and strengthen resistance of ammonia nitrogen stress of Pacific white shrimp. Mohsen et al. [7] found that the addition of 1, 2, and 5 g/kg yeast product in the diet enhanced the growth and protein deposition of Nile tilapia as well as its resistance to *Aeromonas hydrophila* infection. Deng et al. [8] found that dietary 1 and 1.5 g/kg yeast product inclusion reduced intestinal endotoxin levels, enhanced the lysozyme (LSZ) and phenoloxidase (ProPO) activities in shrimp. Furthermore, studies found that yeast product inclusion in the diet enhanced the growth and immunity of pigs [9,10], cows [11], and broilers [12].

Chinese mitten crab (*Eriocheir sinensis*) is an important species in aquaculture and reached 812,103 metric tons in 2016 [13]. Because of its ability to adjust to the environment and high commercial profitability, Chinese mitten crab has been widely cultured in most regions of China [14]. However, with the development of intensive aquaculture, various diseases caused by bacteria [15], viruses [16], and parasites [17] frequently occurred in crab aquaculture and led to culture failure and economic losses [18]. Currently, little is known about the bioavailability of yeast extract applied in Chinese mitten crab. Therefore, this study was carried out to evaluate the effects of dietary yeast extract supplementation on growth, body composition, non-specific immunity, and antioxidant status of Chinese mitten crab.

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Table 1
Measured composition and amino acids profile of yeast extract (dry matter basis, %).

Composition	Content	Amino acid	Content
Moisture	3.4	Lysine (Lys)	4.85
Crude protein	65.3	Leucine (Leu)	4.35
Crude ash	7.9	Valine (Val)	3.61
Bacterial count, CFU/g	10	Arginine (Arg)	3.54
		Threonine (Thr)	2.89
		Isoleucine (Ile)	2.70
		Phenylalanine (Phe)	2.59
		Histidine (His)	1.35
		Methionine (Met)	0.99
		Tryptophan (Trp)	0.79
		Cysteine (Cys)	0.75
		Glutamic acid (Glu)	12.42
		Aspartic acid (Asp)	7.21
		Alanine (Ale)	5.57
		Glycine (Gly)	2.98
		Proline (Pro)	3.23
		Serine (Ser)	2.88
		Tyrosine (Tyr)	2.25

2. Materials and methods

2.1. Yeast extract

The yeast extract (Prosaf®632) was provided by Phileo Lesaffre Animal Care (France). The nutrient components and amino acids profile of yeast extract are presented in Table 1.

2.2. Animal, diet and procedure

The experimental design and procedures were subject to approval by the Institutional Animal Care and Use Committee of Nanjing Agricultural University (Nanjing, China). Seven days before the test crabs were placed into the tanks, the water was injected and the *Alternanthera philoxeroides* were planted into the tanks. Then the water quality has been tested to meets the requirements of the culture water. Water temperature ranged from 24.0 to 26.0 °C, and pH fluctuated between 7.6 and 8.1. Dissolved oxygen was maintained above 7.0 mg/L during the feeding trial. The ammonia-N was maintained below 0.05 mg/L. The Chinese mitten crabs (initial average weight, 4.62 ± 0.11 g) were provided by Jiangsu Jinkangda Group (China). Crabs were reared in an indoor-tanks circulatory system for 1 week to accustom to the experimental conditions and fed with a basal diet (Table 2) prior to the experiment. After the acclimation, a total of 432 crabs with similar size were randomly distributed into 24 plastic tanks (678 L each). The crabs in each tank were randomly assigned one of four experimental diets containing the basal diet supplemented with 0 (control group), 2.5, 5, and 10 g/kg yeast extract, respectively. Each diet was tested in six replicates. The diets were made using a pelleted production line of commercial crab feed in Jiangsu Jinkangda Group (China). Different levels of yeast extract were added into the basal diet in the form of mash and then thoroughly mixed. The mixtures were pelleted (2 mm pellet diameter) in a pellet mill (SDPM420, Shanghai Shende Machinery Co., Ltd, China). All the crabs were hand-fed twice daily at 08:00 and 17:00 with the daily ration of 5% of body weight on a pelleted crab feed to the same location of each tank for 8 weeks. The residual feed were removed by siphoning and then weighted with the air-dry basis every day. The feeding rate was adjusted at every feeding time based on assessment of the leftover feed. 40% of daily diet was fed in the morning and 60% in the nightfall. During the experiment, natural light (no artificial light) cycle was used.

Table 2
Formulation and proximate composition of the basal diets (air-dry basis, %).

Ingredients	Composition	Proximate composition	
Fish meal	22	Moisture	9.50
Shrimp powder	9.6	Crude protein	39.02
Soybean meal	15	Ash	17.03
Rapeseed meal	14	Energy (Mj kg^{-1})	17.45
Cottonseed meal	14		
Wheat flour	20		
Soybean oil	2		
Calcium biphosphate	2		
Sodium chloride	0.4		
Premix[a]	1		

[a] Supplied per kg diet the following minerals and vitamins: Cu, 20 mg; Fe, 50 mg; Mg, 0.2 g; Mn, 15 mg; Zn, 40 mg; Se, 0.16 mg; I, 0.20 mg; Co, 0.25 mg; Vitamin A, 10,000 IU; Vitamin E, 100 mg; Vitamin C, 100 mg; Folic acid, 3 mg; Vitamin D, 2500 IU; Vitamin K_3, 2.2 mg; Vitamin B_1, 3.2 mg; Vitamin B_2, 15 mg; pyridoxine, 10.0 mg; Vitamin B_{12}, 0.016 mg; Choline, 600 mg; Biotin, 0.15 mg; Inositol, 200 mg.

2.3. Sample collections

One male crab from each tank was randomly sacrificed for hemolymph, edible viscera (hepatopancreas and gonads) and abdominal muscle collection at 28 day and 56 day, respectively. The crabs in each tank were anaesthetized on ice for 10 min. The hemolymph of crabs were rapidly taken from the base of the fourth walking-leg using a plastic syringe, then it was placed in a 2 mL eppendorf tube and diluted with an equal volume of anticoagulant solution (2.05 g glucose, 0.8 g citrate and 0.42 g NaCl in 100 mL double distilled water). Edible viscera samples and abdominal muscle were collected and weighted. All the samples were stored at −80 °C for further analysis.

2.4. Growth performance, feed utilization, and biological characteristics

The crabs were weighed before (initial body weight) and after (final body weight) the 8 weeks feeding experiment. The parameters were calculated as follows:

Specific growth rate (SGR, % day^{-1}) = 100 × [ln (final body weight) – ln (initial body weight)] / days

Feed conversion ratio (FCR, g/g) = feed consumed / (final weight - initial weight)

The average number of molting (ANM, times) = total number of molting / number of crabs

Body length width index (BI, m/m) = body length (m) / body width (m)

Edible viscera index (EVI, %) = 100 × edible viscera weight (g) / wet body weight (g)

2.5. Body composition

The samples of edible viscera and muscle were analyzed for crude protein (CP) and crude fat (CF) contents as described by AOAC [19]. The CP (nitrogen × 6.25) content was determined by the Kjeldahl method using an auto Kjeldahl system (FOSS KT260, Hillerød, Denmark). The CF content was determined by ether-extraction using an Auto Soxtec system (FOSS Soxtec™ 2050, Hillerød, Denmark).

2.6. Immune enzyme assay

The ProPO activity was evaluated spectrophotometrically using L-DOPA (3, 4-dihydroxy-L-phenylalanine, Sigma) as the substrate in a 96-well plates [20]. The enzyme activity was measured as the absorbance of dopachrome at 490 nm wavelength using a microplate reader (Thermo Multiskan Go, Thermo Fisher Scientific Inc., USA) for 1 and

3 min, respectively. The ProPO activity was expressed in units defined as the amount of enzyme yielding an increase in absorbance of 0.001 min^{-1}. The LSZ activity was measured by a LSZ assay kit (Nanjing Jiancheng Bioengineering Institute, China). The methodology used in the kit was characterized by Dan et al. [21]. Briefly, the lyophilized *Micrococus lysodeikticus* at a concentration of 0.25 mg/mL was used as a substrate. 0.2 mL of the individual serum or the standard LSZ solution (2.5 μg/mL is equivalent to 200 U/mL) was mixed with 2 mL working solution of *Micrococus lysodeikticus*. The reduction value in absorbance of the reaction solutions were read via a spectrophotometer (UV-1200, Shanghai MAPADA Instruments Co., Ltd, China) at 530 nm by 15 min of the reaction time at 37 °C water. The LSZ activity was calculated refer to the standard lysozyme solution by absorbance. The activities of acid phosphatase (ACP) and alkaline phosphatase (AKP) were determined spectrophotometrically at 520 nm with AKP and ACP detection kit (Nanjing Jiancheng Bioengineering Institute, China), respectively [22]. One unit of AKP activity was defined as the amount of enzyme that reacted with the matrix and produced 1 mg phenol in 30 min at 37 °C. One unit of ACP activity was defined as the amount of enzyme that reacted with the matrix and produced 1 mg phenol in 15 min at 37 °C.

2.7. Antioxidant status assay

Approximately 0.3 g of samples were homogenized (1:9, wt/vol) with ice-cold 154 mmol/L sodium chloride solution using a homogenizer (Pro 200, Pro Scientific Inc., USA), and then centrifuged at 3000 × *g* for 10 min at 4 °C. The catelase (CAT), total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) parameters in the supernatant were measured according to the instructions of the commercial kits (Nanjing Jiancheng Institute of bioengineering, China) [23]. All results were normalized against total protein concentration in each sample for inter-sample comparison. Total protein concentration was determined according to the method of Bradford [24] using bovine serum album as the standard protein.

2.8. Statistical analysis

A one-way ANOVA was conducted for the data using SPSS statistical software (Ver. 20.0 for windows, SPSS Inc., USA). Difference among treatments was examined using the Tukey test. Polynomial contrasts were used to determine the linear and quadratic effects of dietary yeast extract inclusion levels. $P < 0.05$ was used as the threshold for statistical significance. The means and SEM are presented.

3. Result

3.1. Growth performance, feed utilization, and biological characteristics

As indicated in Table 3, dietary supplementation with yeast extract exhibited no significant effects on FBW, SGR, FCR and ANM in Chinese mitten crab ($P > 0.05$). However, compared with the control group, dietary 5 g/kg yeast extract inclusion enhanced the FBW by 5.94%, decreased the FCR by 9.63% in Chinese mitten crab. Table 4 showed that dietary yeast extract inclusion enhanced the EVI at 56 day in Chinese mitten crab (Linear, $P < 0.001$). Dietary 5 and 10 g/kg yeast extract inclusion exhibited higher EVI than that of the control group at 56 day in Chinese mitten crab ($P < 0.05$). Furthermore, although yeast extract inclusion in the diet exhibited no significant difference on BI and EVI at 28 day, dietary 5 g/kg yeast extract inclusion enhanced the 28 day EVI by 22.30% in Chinese mitten crab ($P > 0.05$).

3.2. Body composition

At 56 day, dietary yeast extract inclusion linearly increased the CP content of edible viscera in Chinese mitten crab ($P = 0.025$; Table 5). Dietary supplementation with 2.5, 5, 10 g/kg yeast extract enhanced the CP content of edible viscera at 56 day than that of the control group in Chinese mitten crab by 2.56%, 7.57%, and 13.96%, respectively ($P > 0.05$). The inclusion of yeast extract in the diet of Chinese mitten crab exhibited no significant differences on CF content compared with the control group ($P > 0.05$).

3.3. Immune enzyme assays

Dietary yeast extract supplementation improved the 56 day serum ProPO activity (quadratic, $P = 0.023$) in Chinese mitten crab (Table 6). The addition of 5 g/kg yeast extract in the diet of Chinese mitten crab exhibited higher serum LSZ activity at 28 day and 56 day than that of the control group by 10.08% and 6.76%, respectively ($P > 0.05$). The AKP and ACP activities had no significant difference in Chinese mitten crab among the groups ($P > 0.05$).

3.4. Antioxidant status

Dietary yeast extract inclusion quadratically enhanced the edible viscera T-SOD activity at 28 day ($P = 0.037$; Table 7) and CAT activity at 56 day ($P = 0.034$), increased the 56 day muscular T-SOD activity ($P = 0.020$; Table 8) in Chinese mitten crab. Compared with the control group, the inclusion of 5 g/kg yeast extract in the diet increased the 56 day CAT activity of edible viscera in Chinese mitten crab ($P < 0.05$). The MDA content and T-AOC exhibited no significant difference in Chinese mitten crab among the groups ($P > 0.05$).

4. Discussion

Xiong et al. [25] found that the addition of 10 and 30 g/kg yeast product in the diet exhibited similar FBW, SGR, and FCR with the control group in shrimp. Li et al. [26] reported that dietary supplementation with 20 g/kg yeast product had no significant difference on FBW and feed utilization than that of the control group in shrimp. In this study, dietary 2.5, 5, and 10 g/kg yeast extract inclusion exhibited no significant difference on FBW, SGR, FCR and ANM of Chinese mitten crab among the groups. However, the inclusion of 5 g/kg yeast extract in the diet enhanced the feed utilization by 9.63% than that of the control group. Jin et al. [5] reported that shrimp fed dietary

Table 3
Effects of yeast extract inclusion on the growth performance and feed utilization of Chinese mitten crab (*Eriocheir sinensis*).

Item[a]	Control	Yeast extract (g/kg)			*P* value		
		2.50	5.00	10.00	ANOVA	Linear	Quadratic
IBW (g)	4.90 ± 0.39	4.45 ± 0.07	4.53 ± 0.04	4.59 ± 0.21	0.518	0.396	0.270
FBW (g)	17.52 ± 0.97	18.15 ± 1.70	18.56 ± 1.22	17.86 ± 1.52	0.958	0.819	0.637
SGR ($\%day^{-1}$)	2.29 ± 0.10	2.47 ± 0.20	2.50 ± 0.11	2.40 ± 0.21	0.792	0.609	0.391
FCR (g/g)	1.35 ± 0.12	1.35 ± 0.11	1.22 ± 0.05	1.44 ± 0.21	0.722	0.821	0.419
ANM (times)	1.12 ± 0.07	1.25 ± 0.04	1.18 ± 0.10	1.41 ± 0.19	0.362	0.140	0.692

[a] FBW = final body weight; SGR = specific growth rate; FCR = feed conversion ratio; ANM = the average number of molting.

 Fish and Shellfish Immunology 86 (2019) 1019–1025

Table 4
Effects of yeast extract inclusion on the biological characteristics of Chinese mitten crab (*Eriocheir sinensis*).

Item[1]	Control	Yeast extract (g/kg)			*P* value		
		2.50	5.00	10.00	ANOVA	Linear	Quadratic
28 day							
BI (m/m)	1.12 ± 0.04	1.09 ± 0.02	1.14 ± 0.02	1.07 ± 0.01	0.264	0.396	0.368
EVI (%)	8.61 ± 0.48	9.32 ± 0.46	10.53 ± 0.93	8.90 ± 0.51	0.175	0.467	0.075
56 day							
BI (m/m)	1.10 ± 0.02	1.09 ± 0.01	1.09 ± 0.01	0.93 ± 0.13	0.249	0.100	0.284
EVI (%)	7.71 ± 0.39[b]	9.04 ± 0.33[ab]	9.48 ± 0.31[a]	9.93 ± 0.36[a]	0.001	< 0.001	0.225

Note: Means not sharing the same superscript in each row are significantly different ($P < 0.05$).
[1] BI = body length width index; EVI = edible viscera index.

Table 5
Effects of yeast extract inclusion on the body composition of Chinese mitten crab (*Eriocheir sinensis*).

Item[a]		Control	Yeast extract (g/kg)			*P* value		
			2.50	5.00	10.00	ANOVA	Linear	Quadratic
Edible viscera								
28 day	CP (%)	11.77 ± 0.36	11.25 ± 0.50	10.95 ± 0.79	10.91 ± 0.90	0.793	0.358	0.730
	CF (%)	14.58 ± 1.74	14.30 ± 0.90	14.47 ± 1.41	14.36 ± 1.62	0.999	0.943	0.954
56 day	CP (%)	10.96 ± 0.33	11.24 ± 0.17	11.79 ± 0.58	12.49 ± 0.58	0.135	0.025	0.647
	CF (%)	20.09 ± 1.59	22.51 ± 1.12	22.48 ± 2.99	21.25 ± 1.48	0.784	0.696	0.355
Muscle								
28 day	CP (%)	16.78 ± 1.15	17.76 ± 1.06	16.93 ± 1.47	16.69 ± 0.86	0.909	0.833	0.609
	CF (%)	4.39 ± 0.37	4.13 ± 0.53	4.76 ± 0.46	4.95 ± 0.39	0.566	0.254	0.618
56 day	CP (%)	18.97 ± 1.00	20.19 ± 0.42	19.48 ± 0.96	18.98 ± 1.24	0.781	0.878	0.386
	CF (%)	10.74 ± 0.66	10.16 ± 0.77	11.60 ± 0.76	10.34 ± 1.14	0.645	0.955	0.693

[a] CP = crude protein; CF = crude fat.

supplementation with 10 g/kg yeast hydrolysate or brewer's yeast improved the growth and feed utilization than shrimp fed the control diet. These discrepancies regarding the consequences on the growth and feed utilization of animals may be due to the animal species, properties of yeast product and dosage, and the trial periods. Furthermore, this study found that dietary yeast extract inclusion enhanced the EVI at 56 day in Chinese mitten crab. The better growth and feed utilization in Chinese mitten crab fed dietary yeast extract supplementation could be explained by the rich active ingredients of yeast extract, which could improve the growth of aquatic animal [27–31]. Elsa et al. [3] reported that the yeast extract presented 64% of proteins with high proportion of essential amino acids and 4% of RNA, minerals (such as sodium, 12.28 g/kg; and potassium, 91.48 g/kg; dry weight basis), and B-complex vitamins (such as nicotinic acid, 0.77 g/kg; and pyridoxine, 0.55 g/kg; dry weight basis). Consistently, Deng et al. [8] reported that the supplementation of 1.0 and 1.5 g/kg yeast product in the diet enhanced the FBW and feed utilization of shrimp. Similarly, the improved growth and feed utilization are agreement with the results for white shrimp [32], Nile tilapia [7], rainbow trout [33], and sea bass [34]. The positive effect of yeast extract on growth and feed utilization may contribute by the improved immunity and antioxidant status in Chinese mitten crab.

Yeast extract is a high quality and readily digestible feed ingredients which has been identified as having nutraceutical properties [35]. Previous studies demonstrated that yeast extract could increase the apparent digestibility of nitrogen and individual amino acids in piglets [36]. Mohsen et al. [7] reported that dietary yeast product supplementation increased protein deposition in fish body of Nile tilapia. This study found that yeast extract inclusion in the diet linearly increased the CP accumulation of the edible viscera in Chinese mitten crab. The difference in the CP content of edible viscera may be due to the minerals, B-complex vitamins, and amino acids in yeast extract, such as sodium, potassium, pyridoxine, and lysine [3]. The sodium and potassium are the primary intracellular cations, which are necessary for the activation of the Na^+-K^+-ATPase [37]. Na^+-K^+-ATPase, a key component of extracellular ionic regulation, may benefit for the protein

Table 6
Effects of yeast extract inclusion on the serum immune enzyme assays of Chinese mitten crab (*Eriocheir sinensis*).

Item[a]	Control	Yeast extract (g/kg)			*P* value		
		2.50	5.00	10.00	ANOVA	Linear	Quadratic
28 day							
ProPO (U/min)	0.28 ± 0.03	0.33 ± 0.04	0.33 ± 0.02	0.30 ± 0.03	0.591	0.722	0.193
LSZ (μg/ml)	12.40 ± 0.64	13.43 ± 0.33	13.65 ± 0.26	13.13 ± 0.43	0.230	0.232	0.091
AKP (U/100 mL)	4.71 ± 0.19	5.23 ± 0.32	5.38 ± 0.44	5.38 ± 0.38	0.493	0.181	0.465
ACP (U/100 mL)	5.46 ± 0.30	5.99 ± 0.86	5.75 ± 0.41	5.00 ± 0.46	0.623	0.520	0.258
56 day							
ProPO (U/min)	0.25 ± 0.02	0.32 ± 0.03	0.35 ± 0.03	0.27 ± 0.03	0.112	0.548	0.023
LSZ (μg/ml)	11.99 ± 0.19	12.47 ± 0.16	12.84 ± 0.29	12.49 ± 0.21	0.081	0.067	0.071
AKP (U/100 mL)	4.64 ± 0.18	5.12 ± 0.16	4.90 ± 0.28	5.44 ± 0.71	0.558	0.240	0.944
ACP (U/100 mL)	6.00 ± 0.42	6.80 ± 0.63	6.62 ± 0.35	6.48 ± 0.65	0.743	0.599	0.386

[a] ProPO = phenoloxidase; LSZ = lysozyme; AKP = alkaline phosphatase; ACP = acid phosphatase.

Table 7
Effects of yeast extract inclusion on the antioxidant status of edible viscera in Chinese mitten crab (*Eriocheir sinensis*).

Item[1]	Control	Yeast extract (g/kg)			*P* value		
		2.50	5.00	10.00	ANOVA	Linear	Quadratic
28 day							
MDA (nmol/mg protein)	6.93 ± 0.69	6.45 ± 0.40	6.75 ± 0.68	6.64 ± 0.71	0.957	0.835	0.771
T-SOD (U/mg protein)	43.24 ± 2.15	50.15 ± 2.68	51.10 ± 1.87	46.71 ± 3.18	0.145	0.326	0.037
T-AOC (U/mg protein)	1.60 ± 0.18	1.73 ± 0.21	2.08 ± 0.31	1.91 ± 0.29	0.572	0.272	0.568
CAT (U/mg protein)	0.89 ± 0.06	1.08 ± 0.12	1.15 ± 0.07	1.03 ± 0.09	0.201	0.216	0.079
56 day							
MDA (nmol/mg protein)	5.47 ± 0.62	5.01 ± 0.49	4.74 ± 0.51	5.01 ± 0.48	0.804	0.490	0.500
T-SOD (U/mg protein)	38.41 ± 1.93	38.76 ± 2.90	38.93 ± 2.94	40.35 ± 4.02	0.971	0.664	0.863
T-AOC (U/mg protein)	1.73 ± 0.37	1.73 ± 0.38	1.92 ± 0.31	1.59 ± 0.33	0.924	0.859	0.637
CAT (U/mg protein)	0.83 ± 0.06^{b}	0.95 ± 0.04^{ab}	1.08 ± 0.05^{a}	0.94 ± 0.06^{ab}	0.038	0.077	0.034

Note: Means not sharing the same superscript in each row are significantly different ($P < 0.05$).
[1] MDA = malondialdehyde, T-SOD = total superoxide dismutase, T-AOC = total antioxidant capacity, CAT = catelase.

digestion and absorption in Chinese mitten crab [38]. Liu et al. [39] reported that dietary potassium inclusion enhanced the protein efficiency ratio than that of the control group in shrimp. Wang et al. [27] found that pyridoxine could improve the protein utilization ratio of Chinese mitten crab. Jin et al. [40] reported that the swimming crabs fed the diet containing lysine had higher CP content in the whole body than that fed the control diet. Tovar et al. [41] found yeast product inclusion improved the intestinal trypsin activity of sea bass, which may explain the better protein deposition in Chinese mitten crab. In addition, Hu et al. [42] reported that yeast extract could decrease the concentrations of serum amino acids and urea nitrogen in piglets, which suggested that dietary yeast extract supplementation may increase the amount of amino acids that are available for tissue growth and CP accumulation [43,44]. However, Yuan et al. [45] found that dietary yeast hydrolysate or brewer's yeast supplementation in the practical diets did not affect hepatosomatic index, condition factor, CP, moisture, CF, and crude ash contents in whole body and muscles in juvenile Jian carp. These discrepancies regarding the consequences on the CP contents of animals may be due to the animal species, properties of yeast extract and dosage, and the trial periods. Furthermore, Hassaan et al. [46] reported that dietary 5, 10, and 15 g/kg yeast extract inclusion had no significant difference on body composition of Nile tilapia than that of the control group. Present study found that the addition of yeast extract in the diet had no significant difference on CP and CF content in muscle of Chinese mitten crab among the groups. Similarly, Qiu and Davis [32] reported that the supplementation of yeast product in the practical diets did not affect the moisture, CP, CF, crude fibre, and ash content of whole shrimp body.

The immune and antioxidant systems are two major physiological mechanisms to preserve the health of aquatic animals [47]. Crustacean as Chinese mitten crab have no adaptive immune system that most vertebrate organisms do and instead depends exclusively on the innate immune system to resist various diseases [48,49]. The innate immune system of Chinese mitten crab included cellular and humoral components [50]. The cellular immune response involves encapsulation, phagocytosis and nodule formation, while the humoral response involves a clotting cascade, anti-microbial peptides synthesis and phenoloxidase activating system [50–52]. ProPO, LSZ, ACP, and AKP activities have been applied to evaluate the health condition of crustacean [53]. Jin et al. [5] reported that shrimp fed yeast product in the diet significantly up-regulation the ProPO gene expression levels compared to those fed the control diet. This study found that the supplementation of yeast extract in the diet quadratically increased the 56 day serum ProPO activity of Chinese mitten crab, especially when it is supplemented at 5 g/kg yeast extract in the diet. Furthermore, the addition of 5 g/kg yeast extract in the diet of Chinese mitten crab exhibited higher serum LSZ activities at 28 day and 56 day than that of the control group by 10.08% and 6.76%, respectively. Previous studies found that yeast product could improve the growth performance and immune system of animals [5,11,45,54]. Andrews et al. [55] reported that dietary 10 g/kg yeast extract inclusion enhanced the total leucocyte count and respiratory burst activity of blood in *Labeo rohita*. The containing peptides, nucleotides, vitamins, minerals, and amino acids in yeast extract may contribute to the better immune system. Liu et al. [39] found that dietary supplementation with potassium enhanced the ProPO and LSZ activities than that of the control group in shrimp. Jin et al. [40] reported that dietary lysine inclusion could enhance the health status of hepatopancreas in swimming crabs. Moreover, Sakai et al. [56] reported that the nucleotides from brewers' yeast RNA were capable for enhancing the phagocytic and oxidative activities of kidney phagocytic cells and serum LSZ in common carp as well as resistance to *Aeromonas hydrophila*. Similarly, Deng et al. [8] found that yeast product enhanced

Table 8
Effects of yeast extract inclusion on the antioxidant status of muscle in Chinese mitten crab (*Eriocheir sinensis*).

Item[a]	Control	Yeast extract (g/kg)			*P* value		
		2.50	5.00	10.00	ANOVA	Linear	Quadratic
28 day							
MDA (nmol/mg protein)	1.12 ± 0.14	1.03 ± 0.12	1.04 ± 0.12	0.91 ± 0.09	0.667	0.256	0.844
T-SOD (U/mg protein)	54.27 ± 3.12	65.12 ± 4.32	60.23 ± 3.61	57.47 ± 3.60	0.232	0.778	0.080
T-AOC (U/mg protein)	0.93 ± 0.08	1.02 ± 0.09	0.97 ± 0.11	0.97 ± 0.09	0.922	0.909	0.624
CAT (U/mg protein)	2.56 ± 0.35	2.95 ± 0.40	2.79 ± 0.18	2.57 ± 0.41	0.830	0.943	0.389
56 day							
MDA (nmol/mg protein)	0.79 ± 0.07	0.74 ± 0.06	0.79 ± 0.04	0.71 ± 0.08	0.739	0.544	0.803
T-SOD (U/mg protein)	27.30 ± 1.93	33.80 ± 1.68	35.63 ± 3.91	29.54 ± 1.70	0.102	0.450	0.020
T-AOC (U/mg protein)	0.83 ± 0.12	0.93 ± 0.08	1.07 ± 0.13	1.01 ± 0.10	0.447	0.178	0.468
CAT (U/mg protein)	2.02 ± 0.22	2.21 ± 0.21	2.79 ± 0.31	2.57 ± 0.25	0.155	0.059	0.410

[a] MDA = malondialdehyde, T-SOD = total superoxide dismutase, T-AOC = total antioxidant capacity, CAT = catelase.

the activities of ProPO and LSZ in shrimp.

Free radicals and other reactive species are produced during normal metabolism in cells, which are widely believed to contribute to the development of several diseases by causing oxidative stress and oxidative damage [57]. The SOD has the function to remove the reactive oxygen species from the cytoplasm by catalyzing the dismutation of two superoxide radicals to hydrogen peroxide and oxygen [58]. CAT is a key antioxidant enzyme present in virtually all aerobic organisms that catalyze the breakdown of hydrogen peroxide to water and molecular oxygen [59]. Zhang et al. [6] reported that dietary supplementation with yeast product enhanced the plasma SOD activity in gibel carp with the *Aeromonas hydrophila* challenge. Present study found that dietary yeast extract inclusion could increase the CAT activity of edible viscera, and enhance the SOD activities of edible viscera and muscle in Chinese mitten crab, indicated that yeast extract could improve the anti-oxidative capability of Chinese mitten crab. Similarly, Hu et al. [10] found that the inclusion of yeast extract in the diet improved growth performance and anti-oxidative capability as well as intestinal innate immunity of weaning piglets. The benefit effects on antioxidant status in Chinese mitten crab may result from the rich B-complex vitamins in yeast extract, such as pyridoxine. A cell culture research found that pyridoxine could reduce the level of oxidative stress [60]. Currently, specific mechanisms by which yeast extract and its components influence antioxidative status are unknown. However, Vieira et al. [3] reported that yeast extract had a positive antioxidant activity by Ferric reducing antioxidant potential assay, DPPH (1, 1-Diphenyl-1-Picrylhydrazyl) radical-scavenging capacity assay, and ferricyanide reducing power assay.

5. Conclusion

Dietary yeast extract inclusion increased EVI and the crude protein content of edible viscera, enhanced non-specific immunity, and improved the antioxidant status of edible viscera and muscle in Chinese mitten crab, especially when it is supplemented at 5 g/kg yeast extract in the diet.

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