

## EFFECTS OF DIETARY FUCOIDAN SUPPLEMENTATION ON GROWTH, IMMUNITY, AND DISEASE RESISTANCE IN RED SWAMP CRAYFISH (*Procambarus clarkii*)

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### ABSTRACT

Fucoidan is a polysaccharide that has antioxidant activity, antibacterial activity, antiviral activity, immunomodulatory activity, and hypolipidemic activity. This study aimed to evaluate the impact of fucoidan supplementation on the growth performance, immune response, and disease resistance of Red Swamp crayfish (*Procambarus clarkii*). The effects of different levels of fucoidan supplementation [0 (F0), 1 g/kg (F1), 3 g/kg (F3), and 5 g/kg (F5)] in the diets of Red Swamp crayfish were evaluated. Fucoidan supplementation significantly improved growth performance, immune activity, and disease resistance in the crayfish, with the highest benefits observed at 3% and 5% fucoidan inclusion. A high dosage (F5) did not improve the growth properties compared with a medium dosage (F3) may because of the hypolipidemic activity. Dietary 3 g/kg of fucoidan increased survival rate, body weight gain rate, specific growth rate and food efficiency by 21.51%, 34.51%, 11.43% and 39.39% compared with those of the control group. The optimal dose of dietary fucoidan supplementation required for the maximum growth of crayfish was 3 g/kg. These findings suggest that fucoidan supplementation can be an effective strategy to enhance both the growth and health of Red Swamp crayfish.

**Keywords:** growth, body trophic component, innate immunity.

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### INTRODUCTION

Crayfish are freshwater crustaceans with a wide-range of environmental adaptability, diverse diets, strong fecundity, and high nutrients content. China has the largest aquaculture production of crayfish worldwide (Xiao *et al.*, 2014). Crayfish offers tender, nutritious meat that is high in protein but low in fat, cholesterol, and calories, making it a highly sought-after aquatic product. With the rapid increase in the breeding density and feed intake of crayfish, various problems have begun to appear in the rearing waters and in crayfish well-being, which have resulted in breeding diseases. Excessive use of chemicals and antibiotics can cause a series of problems such as harmful residues and antibiotic resistance; thus, safe and effective materials should be explored to address these issues (Zhang, 2018). In recent years, varying natural materials, such as pectin and *Lactobacillus salivarius* ATCC (Jastaniah *et al.*, 2022), Oak (*Quercus brantii*) acorn extract (Mosafer *et al.*, 2022), *Chlorella vulgaris* (Safari *et al.*, 2022), *Haematococcus pluvialis* (Cai *et al.*, 2022), trehalose (Lu *et al.*, 2021), *Saccharomyces cerevisiae* (Xu *et al.*, 2021), vitamin C (Kong *et al.*, 2021), glycyrrhizic acid (Liu *et al.*, 2020a),

*Codonopsis pilosula polysaccharide* (Liu *et al.*, 2020b), *Rhodiola rosea polysaccharide* (Cheng, 2019), *galactooligosaccharides* (Shiva *et al.*, 2019), chitosan (Sun *et al.*, 2016), fructo-oligosaccharides (Dong and Wang, 2013), L-carnitine (Safari *et al.*, 2015), mannan-oligosaccharides (Safari *et al.*, 2014) and fulvic acid (Zhang, 2018), have been explored to solve this problem.

Fucoidan is a polysaccharide derived from marine algae. It has antioxidant (Wang *et al.*, 2010), antibacterial (Palanisamy *et al.*, 2018), and antiviral activities (Biswajita *et al.*, 2022). It also has immunomodulatory (Elisaveta *et al.*, 2020) and hypolipidemic effects (Ren *et al.*, 2019). Dietary fucoidan has been added to the diets of juvenile common carp (Li *et al.*, 2023), weaned baby goats (Yang *et al.*, 2022), Pacific White Shrimp *Litopenaeus vannamei* (Salehpour *et al.*, 2021), Barramundi *Lates calcarifer* (Bloch) (James *et al.*, 2014), African Catfish (El-Boshy *et al.*, 2014), Giant Tiger Prawn *Penaeus monodon* (Sivagnanavelmurugan *et al.*, 2014), Red Swamp Crayfish *Procambarus clarkii* (Jin *et al.*, 2020), and Yellow Catfish (Yang *et al.*, 2014) to modulate their growth and/or immune response. However, no report has been published on the effect of adding fucoidan to the

feed of crayfish regarding its effect on growth, muscle trophic component, and innate immunity. In view of these data, dietary fucoidan may positive effects on the growth, immunity, and disease resistance of crayfish, which is worth study. Hence, this study aimed to explored the effects of different fucoidan supplementation levels on the growth, immunity, and disease resistance.

## MATERIALS AND METHODS

**Materials:** Fucoidan with a purity  $\geq 95\%$  was obtained from Qingdao Gongying Marine Biotechnology Co., Ltd., Qingdao, China. ELISA kits were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., China.

**Diet preparation:** Referring to previous research (Immanue *et al.*, 2012), when fucoidan is added to the diet at doses of 1, 3, and 5 g/kg, it can have a significant impact on the aquatic animals being raised. Therefore, in this study fucoidan was used to replace 0, 1, 3, and 5 g of cellulose in the basic diet of crayfish (Table 1). All feeds in the experiment were processed by a commercial crayfish extruded- feed pelleting machine (Jiangsu Muyang Company, Changzhou, China)

**Crayfish culture:** Crayfish with a mean body weight of  $0.39 \pm 0.03$  g were supplied by Lianyungang Haishun Aquaculture Co., Ltd, Jiangsu, China. Before feeding trials, crayfish were fed a commercial diet and held for two weeks under experimental conditions. Seven hundred and twenty crayfish were randomly assigned to 12 1,500 L circular tanks equipped with a recirculating aquaculture system. Each group was stocked in triplicate with 60 crayfish per tank. Water variables established for the feeding trials were: temperature  $24 \text{ }^\circ\text{C} \pm 1.5 \text{ }^\circ\text{C}$ ; pH 7.0–8.5; dissolved oxygen  $>5$  mg/L, and photoperiod 12 h dark:12 h light. The crayfish were fed the experimental diet twice daily (08:30 and 17:30). Feed ration was 3% of crayfish body weight daily. At 2 h post-feeding uneaten food and feces were siphoned out of the tanks. The leaching rate of uneaten food is evaluated by placing the weighed food in a water tank without fish for 4 hours, then collecting, drying, and reweighing. The leaching rate is used to calibrate uneaten food. Aerated tap water was supplemented with the daily water exchange rate being  $1/3$ – $2/3$  the total volume.

**Growth and total survival rates:** Survival, feeding, and growth of crayfish were observed daily, and total survival and growth rate were counted were determined after the 8-week culture experiment. Survival (%) =  $100 \times (N_t/N_0)$ ; body weight gain rate (BWGR, %) =  $100 \times (W_t - W_0)/W_0$ ; specific growth rate (SGR, %/day) =  $100 \times (\ln W_t - \ln W_0)/t \times 100$ ; Food efficiency (FE) =  $100 \times (W_t - W_0)/F$ . Where  $N_0$  and  $N_t$  are the numbers of surviving crayfish at the beginning and end of the experimentation;

$W_0$  and  $W_t$  are the crayfish weight at the beginning and end of the experimentation;  $t$  is the number of days of the experimentation, and  $F$  is the total weight of the diet consumed.

**Muscle trophic component assays:** At the end of the feeding experiment, six crayfish were randomly collected from each tank and subjected to proximate analysis in accordance with the AOAC method (AOAC, 2005).

**Innate immune index assay:** At the end of the feeding experiment, six crayfish were randomly collected from each tank, and haemolymph was collected from the pericardial cavity of the head and chest using a 100 disposable medical syringe which specification is 2.5 mL, collect 1 mL per crayfish. The collected haemolymph was transferred to a sterile centrifuge tube, refrigerated at  $5 \text{ }^\circ\text{C}$  for 90 min and centrifuged at  $5,000 \times g$  at  $5 \text{ }^\circ\text{C}$  for 5 min to prepare serum for a biochemical indicator assay. ELISA kits ml022382, ml926247, ml454948, ml918360 and ml076615 were used for the evaluation of the effect of fucoidan included glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), alkaline phosphatase (AKP), and acid phosphatase (ACP) activities, which were determined by using ELISA kits following the instructions of the manufacturer. In addition, total haemocyte count (THC), granular cells (GC), hyaline cells (HC) and semigranular cells (SC) were measured by using a cytometer following the methods of Gullian *et al.* (2004).

**Challenge test:** After the feeding experiment, 30 surviving crayfish were selected randomly from each tank and used for immune response assay. The mortality rate of crayfish was observed during this period. White spot syndrome virus (WSSV) was obtained from the School of Marine Science and Fisheries, Qingdao University of Oceanology, China. A WSSV suspension at  $10^9$ /mL virions/mL was prepared following the methods of Zhang *et al.* (2018). The challenge test was performed by injecting 20  $\mu\text{L}$  of the WSSV suspension containing  $2 \times 10^7$  virions into the last abdominal segment of crayfish. The crayfish were transferred to 100 L tanks containing 80 L of water with a temperature at  $28 \text{ }^\circ\text{C}$ , which was renewed daily. Mortality was recorded every every day after 2 days of injection to calculate cumulative mortality. The challenge test lasted fifteen days.

**Statistical analysis:** All experiments were performed in sextuplicate. Results are presented as the mean  $\pm$  SD ( $n = 6$ ). One-way ANOVA and Duncan's multiple comparisons were performed on the experimental data using SPSS 22.0 statistical software to test the differences among groups. Duncan's multiple-range test was used to determine significant differences, and  $p \leq 0.05$  indicated significant differences.

## RESULTS AND DISCUSSION

**Survival and growth rates:** Fucoidan improved the growth properties of weaned kids (Yang *et al.*, 2022), shrimp *L. vannamei* (Salehpour *et al.*, 2021), *L. calcarifer* (Bloch) (James *et al.*, 2014), African catfish (*C. gariepinus*) (El-Boshy *et al.*, 2014), *P. monodon* (Sivagnanavelmurugan *et al.*, 2014), yellow catfish (*P. fulvidraco*) (Yang *et al.*, 2014) and juvenile red sea bream (*P. major*) (Mahjabin *et al.*, 2018), however, the effects of fucoidan on the growth properties of crayfish remain unknown. After 8-week feeding trials, crayfish fed with fucoidan had higher survival rate, BWGR, SGR and FE than those fed with F0 diet (Table 3,  $P < 0.05$ ). Especially, dietary 3 g/kg of fucoidan increased survival rate, BWGR, SGR and FE by 21.51%, 34.51%, 11.43% and 39.39% compared with those of the control group. However, a high fucoidan dosage (F5) did not improve the efficiency compared with a medium fucoidan dosage (F3). Fucoidan has immunoregulatory and antimicrobial activities; it inhibits microbial growth and decreases microbial infections, thereby improving the survival rate and growth properties of crayfish (Biswajita *et al.*, 2022; Elisaveta *et al.*, 2020; Palanisamy *et al.*, 2018). However, a high dose of fucoidan may primarily exhibit hypolipidemic activity and suppress the efficiency compared with a medium dose of fucoidan (fucoidan3) (Ren *et al.*, 2019). The optimal dose of dietary fucoidan supplementation required for the maximum growth of crayfish was 3 g/kg

**Muscle trophic component:** The effects of dietary treatment with fucoidan on the muscle trophic component of crayfish are shown in Table 4. No differences were found in the ash and moisture contents in the muscle of crayfish amongst all groups ( $P > 0.05$ ). Dietary treatment with fucoidan improved muscle protein content and reduced muscle lipid content compared with those fed with F0 diet ( $P < 0.05$ ). Particularly, treatment with 3g/kg of fucoidan increased muscle protein content by 9.96% and decreased muscle lipid content by 30.98% with those fed with F0 diet. Fucoidan has immunoregulatory and antimicrobial activities, and it inhibits the microbial growth and decreases microbial infections, thereby increasing the physique and amounts of exercise of crayfish as well as muscle protein contents compared with the controls (Biswajita *et al.*, 2022; Elisaveta *et al.*, 2020; Palanisamy *et al.*, 2018). In addition, fucoidan has hypolipidaemic activity and decreased lipid contents compared with the controls (Ren *et al.*, 2019).

**Immunity indicators:** The activities of GPx, SOD, CAT, AKP and ACP and the amounts of THC, GC, HC and SC were used to assess the health of aquatic animals (Chen and Zhang, 2019; Chen and Chen 2019; Cheng and Wu, 2019; Gao *et al.*, 2020; Pan *et al.*, 2018; Zhang, 2019). Changes in the activities of GPx, SOD, CAT, AKP and

ACP and the amounts of THC, GC, HC and SC of crayfish after 8-week of feeding are shown in Table 5. Dietary treatment with fucoidan increased the serum activities of GPx, SOD, CAT, AKP and ACP and the amounts of THC, GC, HC and SC compared with those of crayfish fed with F0 diet ( $P < 0.05$ ). Typically, treatment with 3g/kg of fucoidan increased the serum activities of GPx, SOD, CAT, AKP and ACP by 49.70%, 67.37%, 42.99%, 34.83% and 24.28% and the amounts of THC, GC, HC and SC by 74.19%, 109.32%, 139.47% and 133.33% compared with those of crayfish fed with F0 diet. The increase in SOD and GPx activities may be ascribed to the antioxidant activity of fucoidan (Wang *et al.*, 2010), whilst the increase in AKP and ACP activities as well as THC, HC, SC and GC may be ascribed to the immunomodulatory activity of fucoidan (Wang *et al.*, 2010). Similarly, dietary treatment with fucoidan stimulated the immune response of weaned kids (Yang *et al.*, 2022), shrimp *L. vannamei* (Salehpour *et al.*, 2021), *L. calcarifer* (Bloch) (James *et al.*, 2014), African catfish (*C. gariepinus*) (El-Boshy *et al.*, 2014), *P. monodon* (Sivagnanavelmurugan *et al.*, 2014), yellow catfish (*P. fulvidraco*) (Yang *et al.*, 2014) and juvenile red sea bream (*P. major*) (Mahjabin *et al.*, 2018).

**Table 1. Composition of supplementing fucoidan for cellulose in the diets of the Red Swamp crayfish diets (g kg diet<sup>-1</sup>).**

Ingredient (g kg <sup>-1</sup> )	F0	F1	F3	F5
Fish meal	420	420	420	420
Wheat flour	350	350	350	350
crayfish shell meal	70	70	70	70
Soybean meal	70	70	70	70
Gluten	25	25	25	25
Yeast meal	25	25	25	25
Fish oil	8	8	8	8
Cellulose	6	5	3	1
Fucoidan	0	1	3	5
Mineral mixture <sup>a</sup>	20	20	20	20
Vitamin mixture <sup>b</sup>	6	6	6	6
Proximate composition				
Protein	294	294	294	294
Lipid	68	68	68	68
Ash	71	71	71	71
Energy (MJ kg <sup>-1</sup> )	187	187	187	187

<sup>a</sup>Vitamin mixture (g kg diet<sup>-1</sup>):  $\beta$ -Carotene, 3 M.I.U.; Cholecalciferol, 0.6 M.I.U.; Thiamin, 3.6; Riboflavin, 7.2; Pyridoxine, 6.6; Cyanocobalamine, 0.02;  $\alpha$ -Tocopherol, 16.5; Menadione, 2.4; Niacin, 14.4; Pantothenic acid, 4; Biotin, 0.02; Folic acid, 1.2; Inositol, 30; Ascorbic acid, 100.

<sup>b</sup>Mineral premix (g kg diet<sup>-1</sup>): P, 120; Ca, 120; Mg, 15; Fe, 1.5; Zn, 4.2; Cu, 2.1; K, 75; Co, 0.11; Mn, 1.6; Se, 0.01; Mo, 0.005; Al, 0.025; I, 0.4.

**Challenge test:** Crayfish mortalities occurred after 3 days of injection with WSSV in the control group and

groups fed fucoidan-containing diets. The cumulative mortality of crayfish fed with fucoidan-containing diets was lower than that of crayfish fed with F0 diet during the challenge test ( $P < 0.05$ , Table 7). At the end of 15 days of the challenge test, the cumulative mortality of that of crayfish fed with F0 diet reached up to 52.63%, whilst the cumulative mortality of that of crayfish fed F3

diet was only 15.37%. Dietary fucoidan increased the numbers of GC, THC, HC and SC, thereby decreasing the WSSV infection. The results could be due to the antibacterial, antiviral and immunomodulatory activities of fucoidan (Biswajita *et al.*, 2022; Elisaveta *et al.*, 2020; Palanisamy *et al.*, 2018).

**Table 2. Body weight gain rate (BWGR), specific growth rate (SGR), feed efficiency (FE), and survival of Red Swamp crayfish, after 8 weeks of feeding diets supplemented with fucoidan. Supplementation amounts (F) were expressed as g/Kg.**

Parameters	F0	F1	F3	F5
Crude protein (%)	75.32±6.13 <sup>a</sup>	82.41±7.26 <sup>b</sup>	91.52±6.27 <sup>c</sup>	92.25±5.74 <sup>c</sup>
Crude lipids (%)	950.16±16.37 <sup>a</sup>	1067.38±18.82 <sup>b</sup>	1278.07±20.13 <sup>c</sup>	1271.32±21.07 <sup>c</sup>
Moisture (%)	4.20±0.02 <sup>a</sup>	4.38±0.03 <sup>a</sup>	4.68±0.05 <sup>b</sup>	4.66±0.04 <sup>b</sup>
Ash (%)	0.33±0.03 <sup>a</sup>	0.36±0.05 <sup>a</sup>	0.46±0.06 <sup>b</sup>	0.45±0.06 <sup>b</sup>

The different superscript letters indicate significant differences for each row ( $P < 0.05$ ). Values are the mean ± SD (n = 6).

**Table 3. Muscle proximate composition of red swamp crayfish, after 8 weeks of feeding the experimental diets.**

Parameters	F0	F1	F3	F5
Crude protein (%)	9.04±0.32 <sup>a</sup>	9.86±0.35 <sup>b</sup>	9.94±0.41 <sup>b</sup>	10.03±0.39 <sup>b</sup>
Crude lipids (%)	3.26±0.21 <sup>a</sup>	2.44±0.22 <sup>b</sup>	2.27±0.25 <sup>b</sup>	2.25±0.19 <sup>b</sup>
Moisture (%)	78.84±1.07 <sup>a</sup>	78.91±1.23 <sup>a</sup>	78.72±1.07 <sup>a</sup>	78.75±1.37 <sup>a</sup>
Ash (%)	5.81±0.12 <sup>a</sup>	5.92±0.13 <sup>a</sup>	5.79±0.16 <sup>a</sup>	5.86±0.18 <sup>a</sup>

The different superscript letters indicate significant differences for each row ( $P < 0.05$ ). Values are the mean ± SD (n = 6).

**Table 4. Glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), alkaline phosphatase (AKP) and acid phosphatase (ACP) activities and total haemocyte count (THC) and number of granular cells (GC), hyaline cells (HC), semigranular cells (SC) after 8 weeks of feeding the experimental diets.**

Parameters	F0	F1	F3	F5
GPx (U g protein <sup>-1</sup> )	31.21±3.24 <sup>a</sup>	37.42±3.76 <sup>b</sup>	46.72±4.71 <sup>c</sup>	47.15±5.13 <sup>c</sup>
SOD (U mg protein <sup>-1</sup> )	21.18±1.03 <sup>a</sup>	27.12±1.17 <sup>b</sup>	35.45±1.56 <sup>c</sup>	36.16±1.46 <sup>c</sup>
CAT (U g protein <sup>-1</sup> )	16.33±1.62 <sup>a</sup>	19.26±1.81 <sup>b</sup>	23.35±2.03 <sup>c</sup>	24.19±2.16 <sup>c</sup>
AKP (U g protein <sup>-1</sup> )	43.37±1.26 <sup>a</sup>	48.31±1.31 <sup>b</sup>	57.61±1.82 <sup>c</sup>	59.35±1.94 <sup>c</sup>
ACP (U g protein <sup>-1</sup> )	93.51±4.17 <sup>a</sup>	105.31±5.65 <sup>b</sup>	116.21±5.83 <sup>c</sup>	117.26±6.04 <sup>c</sup>
THC (×10 <sup>4</sup> mL <sup>-1</sup> )	3.41±0.19 <sup>a</sup>	4.31±0.21 <sup>b</sup>	5.94±0.24 <sup>c</sup>	6.07±0.19 <sup>c</sup>
GC (×10 <sup>4</sup> mL <sup>-1</sup> )	1.18±0.08 <sup>a</sup>	1.39±0.21 <sup>b</sup>	2.47±0.32 <sup>c</sup>	2.53±0.33 <sup>c</sup>
HC (×10 <sup>4</sup> mL <sup>-1</sup> )	1.51±0.09 <sup>a</sup>	2.39±0.21 <sup>b</sup>	3.62±0.11 <sup>c</sup>	3.71±0.12 <sup>c</sup>
GC (×10 <sup>4</sup> mL <sup>-1</sup> )	0.42±0.03 <sup>a</sup>	0.67±0.05 <sup>b</sup>	0.98±0.08 <sup>c</sup>	1.03±0.07 <sup>c</sup>

The different superscript letters indicate significant differences for each row ( $P < 0.05$ ). Values are the mean ± SD (n = 6).

**Table 5. Cumulative mortality (%) of red swamp crayfishes fed with experimental diets, after being challenged by pathogen WSSV.**

Time after challenge (d)	F0	F1	F3	F5
5	18.9±0.68 <sup>a</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>
10	46.3±1.33 <sup>a</sup>	9.06±0.41 <sup>b</sup>	8.16±0.31 <sup>b</sup>	7.76±6.5 <sup>b</sup>
15	52.63±1.94 <sup>a</sup>	16.14±0.73 <sup>b</sup>	15.37±0.61 <sup>b</sup>	14.86±0.63 <sup>b</sup>

The different superscript letters indicate significant differences for each row ( $P < 0.05$ ). Values are the mean ± SD (n =

**Conclusions:** Dietary treatment with fucoidan improved the growth properties, muscle trophic component, immune response and immunity-related gene expression

of red swamp crayfish *P. clarkii*. Nevertheless, a high fucoidan dosage (F5) did not further increase the efficiency. The results indicated that fucoidan could be used as a diet supplement to improve the growth properties, body trophic component and innate immunity of crayfish.

**Ethics Statement:** This study was approved by the ethics committee of Jiangsu Ocean University of Technology, China. All procedures were conducted in compliance with relevant laws and institutional guidelines.

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