

## Original Research Articles

# Comparative Analysis of Growth Traits, Textural Attributes, and Blood Biochemical Parameters in *Procambarus clarkii* under Varied Feeding Regimens

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To systematically evaluate the trophic effects of *Elodea nuttallii* as exclusive versus complementary feedstock on growth performance, textural properties, and biochemical profiles in *Procambarus clarkii*, we conducted a controlled feeding trial employing four distinct nutritional regimes. The experiment followed a completely randomized design with four treatments (n=3 replicates/treatment; 20 crayfish/replicate). Dietary treatments included: a control group (0#) with access only to *E. nuttallii* and no supplemental feed, a soybean-supplemented group (1#) with *E. nuttallii*, a corn-supplemented group (2#) with *E. nuttallii*, and a formulated feed-supplemented group (3#) with *E. nuttallii*. Crayfish were fed twice daily (07:00 and 17:00) for 6 weeks. Results indicated that the control group (0#) showed significantly lower weight gain (WG) versus supplemented groups ( $P < 0.05$ ) and the 3# experimental group showed the best growth. Among the four experimental groups, no significant differences were observed in hardness, brittleness, or elasticity of the muscle ( $P > 0.05$ ). However, muscle cohesiveness peaked in the corn-supplemented group, differing significantly from other treatments ( $P < 0.05$ ). In terms of gumminess, the 1# group demonstrated the highest value (8.50), which was significantly greater than that of the other groups ( $P < 0.05$ ). Total protein (TP) reached maximum levels in the formulated feed group (3#), being significantly higher than in groups 0# and 2# ( $P < 0.05$ ) but statistically equivalent to group 1# ( $P > 0.05$ ). In contrast, the control group (0#) recorded the lowest TP content at 12.60. Regarding GLB (globulin) content, the 1#, 2#, and 3# groups demonstrated significantly higher values compared to the control group ( $P < 0.05$ ). However, no significant differences in GLB content were observed among these three groups ( $P > 0.05$ ). The results of this study suggest that *P. clarkii* exhibited optimal growth performance when reared with formulated feed supplemented with *E. nuttallii*. The soybean-*E. nuttallii* dietary combination yielded superior muscle nutritional quality and organoleptic characteristics. Plasma biochemical analyses confirmed the aquacultural advantages when *Elodea*-based systems were complemented with protein-rich feed supplementation, with the formulated feed regime showing particular promise.

## INTRODUCTION

Crayfish, *Procambarus clarkii*, is one of the larger freshwater shrimp species. Renowned for their delectable taste, cray-

fish is esteemed as a nutritious delicacy, boasting high levels of muscle protein and calcium, coupled with low fat content. In recent years, China's consumer market for crayfish has kept expanding, while the aquaculture industry has

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progressed at an unparalleled rate. In China, the total aquaculture production of crayfish in 2023 reached 3.16 million tons, with a 9.35% increase from 2022. As a result, crayfish has become the predominant crustacean species cultivated in China.

Crayfish demonstrate exceptional thermal adaptability, flourishing in water temperatures that span from 10 to 30°C. Under optimal conditions of temperature and abundant feed, it can reach the marketable size within 40-50 days. Its dietary preferences vary across developmental stages, environmental conditions, and individual size, showcasing a broad trophic spectrum. In natural habitats, crayfish primarily feed on detritus, organic debris, aquatic vegetation, algae, zooplankton, aquatic insects, benthic organisms, and animal carcasses. However, under conditions of feed scarcity, cannibalistic behavior may occur.<sup>1</sup>

In recent years, the escalating consumer demand for crayfish, driven by rising living standards, has spurred the proliferation of diverse integrated aquaculture models, including rice-crayfish co-culture, lotus pond cultivation, pond monoculture, and polyculture with fish. While numerous studies have explored the nutritional quality of crayfish muscle across these farming systems,<sup>2-4</sup> comparative analyses of growth performance, textural properties, and blood biochemical profiles under varying feed regimes within identical ecological settings remain scarce.

*Elodea nuttallii* is characterized by its cylindrical, fragile stems. Esteemed for its nutritional richness, freshness, and tender texture, *E. nuttallii* serves as an excellent natural feed for aquatic species such as crabs and crayfish. Therefore, this study aims to systematically compare the general composition, textural characteristics, and blood biochemical indices of crayfish reared under identical water quality and aquatic vegetation conditions, with access to *E. nuttallii* and supplemented diets such as soybean, corn, and formulated feed. The findings are expected to provide valuable theoretical insights into crayfish aquaculture.

## MATERIALS AND METHODS

### EXPERIMENTAL ANIMALS AND DESIGN

*P. clarkii* juveniles used in this feeding trial were procured from Yuanlv Agriculture Co., Ltd. (yutai, Shandong Province, P.R China). Firstly, *P. clarkii* were cultured in a tank (500L) to acclimate to the experimental conditions by feeding the commercial diet twice daily (07:00 and 17:00) for one week. After the conditioning period, a total of 240 *P. clarkii* with similar sizes (initial weight =  $4.46 \pm 0.93$  g) were randomly divided into 12 fiberglass tanks ( $1.5 \times 1 \times 1$  m). The experiment was arranged in a completely randomized design comprising 4 treatment groups with 3 replicates per group and 20 crayfish per replicate. Four groups were studied: a control group (0<sup>#</sup>) with access only to *E. nuttallii* and no supplemental feed, a soybean-supplemented group (1<sup>#</sup>) with *E. nuttallii*, a corn-supplemented group (2<sup>#</sup>) with *E. nuttallii*, and a formulated feed-supplemented group (3<sup>#</sup>) with *E. nuttallii*. Crayfish were fed twice daily at 07:00 and 17:00, respectively, for 6 weeks. To create a tranquil and

shaded environment conducive to crayfish growth, black polyethylene mesh was suspended above the tanks to block sunlight. Additionally, 20 wide-mouth glass or plastic bottles filled with pond mud were placed in each tank to provide hiding spaces. *E. nuttallii* was planted in clusters of 10, with row and plant spacings of approximately 30 cm and 20 cm, respectively. Furthermore, *E. nuttallii* stems measuring 10-20 cm in length were scattered across the tanks, achieving a coverage rate of approximately two-thirds. *E. nuttallii* coverage was replenished promptly whenever it fell below one-third of the tank area. Corn and soybeans were boiled before feeding, and the daily feeding rate was set at 5 % of the crayfish's body weight.

### FEEDING MANAGEMENT

During the whole feeding trial, the mortality, molting times and feed intake were recorded every day. The water source for the experiment was 700 m deep mineral water. Water temperature ( $25 \pm 1^\circ\text{C}$ ), dissolved oxygen ( $4.70 \pm 0.8$  mg/L), pH ( $7.25 \pm 0.23$ ) and ammonia-nitrogen ( $0.11 \pm 0.02$ ) maintained within the acceptable limits for the culture of this species.

### SAMPLE COLLECTION

At the end of feeding trial, *P. clarkii* were starved for 24 h before sampling. Then, all *P. clarkii* in each tank were anesthetized in diluted MS-222 (tricainemethanesulfonate, Sigma, USA) at a concentration of 80 mg/L and measured for final weight. Five individuals from each tank were randomly selected to collect hemolymph with 1.0 mL sterile syringes in 1.5 mL Eppendorf tubes and then stored at 4 °C for subsequent analyses.

### CALCULATION OF GROWTH AND MOLTING PERFORMANCE

For each treatment, all *P. clarkii* were determined to quantify the final weight, percent of weight gain, feed intake, feed efficiency, survival rate, molting times, molting rate and weekly molting times during the whole feeding trial. The growth and molting parameters were calculated as follows:

$$\text{Survival rate, SR} = 100\% \times (K_t/K_0)$$

$$\text{Weight gain, WG} = 100\% \times (W_t - W_0)/W_0$$

$$\text{Specific growth rate, SGR} = 100\% \times (\ln W_t - \ln W_0)/t$$

$$\text{Feed conversion ratio, FCR} = 100\% \times G_d/(W_t - W_0)$$

$$\text{Feed intake (FI, g/g of crayfish/day)} = \text{feed consumption}/((W_t + W_0)/2 \times \text{the experimental duration in days})$$

$$\text{Meat content, MC} = 100\% \times G_m/W_t$$

where,  $K_t$  is the final number of crayfish,  $K_0$  is the initial number of crayfish,  $W_t$  is the final weight,  $W_0$  is the initial weight, it is the experimental days,  $G_d$  is the food ration,  $L$  is the final body length, and  $G_m$  is the muscle weight.

### DETERMINATION OF WHOLE-BODY COMPOSITION

The conventional components of test feed and whole crayfish were determined according to the method described by

You et al.<sup>5</sup> Moisture was determined by the constant weight drying method at 80 °C, crude lipid content by Soxhlet extraction method, and crude protein ( $N \times 6.25$ ) using the Kjeldahl method. The sample was burned in a muffle furnace at 550 °C for 6 h, and the crude ash content was determined by the carbonization method.

#### MEASUREMENT OF MUSCLE TEXTURAL PROPERTIES

Muscle samples were randomly collected from five crayfish to evaluate textural properties. Texture profile analysis (TPA) was conducted using a Universal TA texture analyzer to determine parameters such as hardness, brittleness, elasticity, cohesiveness, gumminess, and chewiness. Each sample was measured five times to ensure data reliability.

#### ANALYSIS OF PLASMA BIOCHEMICAL PARAMETERS

Blood samples were centrifuged at 5,000 rpm for 10 minutes, and the supernatant was stored at -20°C for the subsequent analysis. Plasma biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), albumin (ALB), triglycerides (TG), cholesterol (TC), and glucose (GLU), were quantified using a BS-430 fully automated biochemical analyzer.

#### STATISTICAL ANALYSIS

The experimental data were analyzed using SPSS 25.0 for biological statistics. The assumptions of normality and mean square deviation were confirmed before any statistical analysis. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to determine whether different levels of substitution had a significant effect on the measurements. The level of significance was set as  $P < 0.05$ . Statistics were expressed as mean  $\pm$  SEM (standard error of the mean) and differences were considered significant at  $P < 0.05$ . Principal component analysis and plotting using origin 2018.

## RESULTS

#### GROWTH PERFORMANCE OF CRAYFISH UNDER DIFFERENT FEEDING REGIMES

As illustrated in [Table 1](#), after 6 weeks of feeding, the control group (0<sup>#</sup>) exhibited a markedly slower increase in body weight compared to other three groups ( $P < 0.05$ ). The 3<sup>#</sup> experimental group showed the best growth. However, the WG of the 3<sup>#</sup> group did not significantly differ from that of the 1<sup>#</sup> and 2<sup>#</sup> groups ( $P > 0.05$ ). The SGR was lowest in the 0<sup>#</sup> group at ( $2.25 \pm 0.19$ )%, while the 3<sup>#</sup> group achieved the highest SGR of ( $5.43 \pm 0.31$ )%. SGR followed the hierarchy: 3<sup>#</sup> > 1<sup>#</sup> > 2<sup>#</sup> > 0<sup>#</sup>, showing significant intergroup differences ( $P < 0.05$ ). Muscle yield peaked in the control group ( $17.20 \pm 0.36$ %), being significantly higher than supplemented groups ( $P < 0.05$ ). In contrast, no significant difference in meat yield was observed among the 1<sup>#</sup>, 1<sup>#</sup>, and 3<sup>#</sup> groups ( $P > 0.05$ ). Survival rates varied considerably

among the groups, with the 3<sup>#</sup> group exhibiting the highest survival rate of ( $85.00 \pm 6.12$ )%, while the 2<sup>#</sup> group recorded the lowest at ( $56.67 \pm 4.75$ )%. Significant differences in survival rates were noted across all groups ( $P < 0.05$ ).

#### EFFECTS OF DIFFERENT FEEDING REGIMES ON THE PROXIMATE COMPOSITION OF CRAYFISH MUSCLE

The moisture content of crayfish muscle exhibited minimal variation across the experimental groups ([Table 2](#)). The 0<sup>#</sup> group displayed a slightly higher moisture content of  $81.28 \pm 0.72$  %, while the 3<sup>#</sup> group recorded the lowest at  $79.89 \pm 0.33$  %. However, no statistically significant difference was observed among the groups ( $P > 0.05$ ). In terms of crude protein content, the 0<sup>#</sup> group showed the lowest value at  $16.46 \pm 0.11$  %, which was significantly lower compared to the other three groups ( $P < 0.05$ ). The highest crude protein content was observed in the 3<sup>#</sup> group at  $18.09 \pm 0.21$  %, though this did not differ significantly from groups 1<sup>#</sup> and 2<sup>#</sup> ( $P > 0.05$ ).

The crude fat content was lowest in the 0<sup>#</sup> group ( $0.68 \pm 0.05$  %), showing significant differences compared to the 2<sup>#</sup> and 3<sup>#</sup> groups ( $P < 0.05$ ). Conversely, the 3<sup>#</sup> group exhibited the highest crude fat content at  $0.9 \pm 0.04$  %, which was significantly higher than the 1<sup>#</sup> group ( $P < 0.05$ ) but not significantly different from the 2<sup>#</sup> group ( $P > 0.05$ ). Regarding ash content, the 3<sup>#</sup> group had the highest value at  $1.54 \pm 0.15$  %, while the 0<sup>#</sup> group recorded the lowest at  $1.43 \pm 0.11$  %. However, no significant differences in ash content were detected among the groups ( $P > 0.05$ ).

#### EFFECTS OF DIFFERENT FEEDING REGIMES ON THE TEXTURAL PROPERTIES OF CRAYFISH MUSCLE

Textural analysis revealed significant diet-induced variations in *Procambarus clarkii* muscle characteristics ([Table 3](#)). Among the four experimental groups, no significant differences were observed in hardness, brittleness, or elasticity of the muscle ( $P > 0.05$ ). However, the cohesiveness of muscle was highest in the 2<sup>#</sup> group (1.80), significantly surpassing the other groups ( $P < 0.05$ ). In terms of gumminess, the 1<sup>#</sup> group demonstrated the highest value (8.50), which was significantly greater than that of the other groups ( $P < 0.05$ ). Conversely, the formulated feed group (3<sup>#</sup>) showed minimal gumminess (7.28), differing significantly from groups 1<sup>#</sup> and 2<sup>#</sup> ( $P < 0.05$ ). Regarding chewiness, the 0<sup>#</sup> and 3<sup>#</sup> groups recorded the lowest values, which were significantly lower than that of the 1<sup>#</sup> group ( $P < 0.05$ ) but not significantly different from the 2<sup>#</sup> group ( $P > 0.05$ ). The 1<sup>#</sup> group achieved peak chewiness (8.72), being significantly superior to groups 0<sup>#</sup> and 3<sup>#</sup> ( $P > 0.05$ ) but statistically equivalent to group 2<sup>#</sup> ( $P > 0.05$ ).

#### EFFECTS OF DIFFERENT FEEDING REGIMES ON BLOOD BIOCHEMICAL PARAMETERS OF CRAYFISH

As shown in [Table 4](#), no significant differences were observed in serum biochemical markers, including AST, ALT, AKP, Glu, TG, and TC, across the experimental groups ( $P > 0.05$ ). However, the TP content exhibited notable varia-

**Table 1. Growth performance of crayfish under different feeding regimes**

	0 <sup>#</sup>	1 <sup>#</sup>	2 <sup>#</sup>	3 <sup>#</sup>
Final weight, FW (g)	8.26±1.79 <sup>a</sup>	15.59±2.07 <sup>b</sup>	14.30±2.46 <sup>b</sup>	17.93±3.54 <sup>b</sup>
Final body length, FBL (cm)	5.73±0.47 <sup>a</sup>	6.62±0.83 <sup>b</sup>	6.52±0.97 <sup>b</sup>	7.16±1.31 <sup>b</sup>
Specific growth rate, SGR (%)	2.25±0.19 <sup>a</sup>	4.90±0.34 <sup>bc</sup>	4.53±0.59 <sup>b</sup>	5.43±0.31 <sup>c</sup>
Meat content, MC (%)	17.20±0.36 <sup>b</sup>	15.37±0.17 <sup>a</sup>	14.26±0.41 <sup>a</sup>	14.99±0.36 <sup>a</sup>
Survival rate, SR (%)	68.33±3.29 <sup>b</sup>	76.67±5.04 <sup>c</sup>	56.67±4.75 <sup>a</sup>	85.00±6.12 <sup>d</sup>

Note: Data are expressed as means ± SEM (n = 9). Diverse small letters show significant differences (P<0.05) in different dosage groups.

**Table 2. Effects of Different Feeding Regimes on the Proximate Composition of Crayfish Muscle**

	0 <sup>#</sup>	1 <sup>#</sup>	2 <sup>#</sup>	3 <sup>#</sup>
Moisture (%)	81.28±0.72	80.01±0.58	80.25±0.43	79.89±0.33
Crude Protein, CP (%)	16.46±0.11 <sup>b</sup>	17.94±0.12 <sup>b</sup>	17.44±0.15 <sup>a</sup>	18.09±0.21 <sup>b</sup>
Crude Fat, CF (%)	0.68±0.05 <sup>a</sup>	0.81±0.08 <sup>ab</sup>	0.87±0.11 <sup>b</sup>	0.9±0.04 <sup>b</sup>
Crude Ash, CA (%)	1.43±0.11	1.52±0.09	1.46±0.09	1.54±0.15

Note: Data are expressed as means ± SEM (n = 9). Diverse small letters show significant differences (P<0.05) in different dosage groups.

**Table 3. Effects of Different Feeding Regimes on the Textural Properties of Crayfish Muscle**

	0 <sup>#</sup>	1 <sup>#</sup>	2 <sup>#</sup>	3 <sup>#</sup>
hardness	5.06±0.11	5.07±0.11	5.04±0.09	5.04±0.08
brittleness	4.55±0.17	4.64±0.16	4.59±0.19	4.32±0.18
elasticity	0.99±0	0.99±0	0.99±0	0.99±0
cohesiveness	1.46±0.15 <sup>a</sup>	1.50±0 <sup>a</sup>	1.80±0.14 <sup>b</sup>	1.43±0.29 <sup>a</sup>
gumminess	7.49±0.21 <sup>ab</sup>	8.50±0.35 <sup>c</sup>	7.78±0.40 <sup>b</sup>	7.28±0.24 <sup>a</sup>
chewiness	7.01±0.23 <sup>a</sup>	8.72±0.32 <sup>b</sup>	7.91±0.70 <sup>ab</sup>	7.20±0.41 <sup>a</sup>

Note: Data are expressed as means ± SEM (n = 9). Diverse small letters show significant differences (P<0.05) in different dosage groups.

tions. The 3<sup>#</sup> group displayed the highest total protein level at 19.75, significantly exceeding that of groups 0<sup>#</sup> and 2<sup>#</sup> (P < 0.05), though no significant difference was observed compared to the 1<sup>#</sup> group (P > 0.05). In contrast, the control group (0<sup>#</sup>) recorded the lowest total protein content at 12.60, which was significantly lower than that of the other experimental groups (P < 0.05). GLB content was significantly elevated in protein-supplemented groups (1<sup>#</sup>-3<sup>#</sup>) relative to control (P < 0.05). However, no significant differences in globulin content were observed among these three groups (P > 0.05).

## DISCUSSION

The growth performance of *P. clarkii* is influenced by multiple factors, including biological characteristics, food resources, developmental stages, stocking density, and environmental conditions. In this study, comparison of different diets revealed that the formulated feed group exhibited the most favorable growth outcomes, followed by the soybean and corn groups, while the control group showed the slowest growth. These results suggest that formulated feed better meets the energy and nutritional requirements of crayfish, promoting rapid growth. The com-

prehensive nutritional profile of formulated feed, providing a balanced supply of proteins, lipids, carbohydrates, and micronutrients, is crucial for growth, molting, and immune function maintenance in crayfish.<sup>6</sup> Additionally, the significantly lower consumption of *E. nuttallii* in the formulated feed group indicates reduced reliance on natural forage, further supporting its nutritional adequacy. Moreover, the higher survival rate in this group suggests that the feed's composition mitigates mortality caused by molting failure or cannibalism.

However, the water quality in the formulated feed group deteriorated more rapidly, likely due to the accumulation of uneaten feed residues and excretory waste. Although darker water may provide some shelter for crayfish, regular water exchange is essential to ensure normal growth.<sup>7</sup> Among the experimental groups, the corn group exhibited the poorest growth performance. Although its specific growth rate and final body weight were higher than those of the control group, residual corn kernels were frequently observed, indicating poor palatability. Moreover, the significantly lower survival rate in this group may be attributed to nutritional imbalances and increased cannibalism.<sup>8</sup> In contrast, the control group showed a significantly higher meat yield, likely due to the smaller body size and thinner exoskeleton

**Table 4. Effects of Different Feeding Regimes on blood Biochemical Parameters of Crayfish**

	0 <sup>#</sup>	1 <sup>#</sup>	2 <sup>#</sup>	3 <sup>#</sup>
Aspartate aminotransferase, AST (U/L)	1.35±0.23	1.62±0.37	1.45±0.25	1.50±0.39
Alanine aminotransferase, ALT (U/L)	1.27±0.20	1.32±0.32	1.00±0.12	1.32±0.23
Alkaline phosphatase, AKP (U/L)	1.95±0.14	2.61±0.72	2.27±0.31	2.32±0.56
Glucose, GLU (mmol/L)	36.93±0.49	37.56±1.56	36.08±1.31	35.23±0.83
Triglycerides, TG (mmol/L)	0.04±0.01	0.05±0.01	0.05±0.01	0.04±0.01
Cholesterol, TC (mmol/L)	0.10±0.03	0.12±0.04	0.11±0.07	0.15±0.01
Total protein, TP (mg/L)	12.60±1.24 <sup>a</sup>	17.98±0.92 <sup>bc</sup>	16.40±1.56 <sup>b</sup>	19.75±2.11 <sup>c</sup>
Albumin, ALB (mg/L)	3.43±0.74	4.38±0.53	3.98±0.46	4.33±0.99
Globulin, GLB (mg/L)	9.17±1.65 <sup>a</sup>	13.6±0.42 <sup>b</sup>	12.42±0.87 <sup>b</sup>	15.42±2.68 <sup>b</sup>

Note: Data are expressed as means ± SEM (n = 9). Diverse small letters show significant differences (P<0.05) in different dosage groups.

of the crayfish. However, this does not reflect superior growth performance, as the slow growth was a result of insufficient nutritional supply.<sup>9</sup> The soybean group exhibited intermediate growth performance, with moderate meat yield and survival rates. While soybeans are a high-quality protein source, its nutritional profile is less comprehensive compared to formulated feed.<sup>10</sup> The lower consumption of *E. nuttallii* in the soybean group compared to the control and corn groups suggests that its nutritional supply, though superior to corn, still falls short of fully meeting crayfish growth requirements.

The proximate composition of muscle, including moisture, protein, fat, and ash content, directly determines the economic and nutritional value of crayfish. Variations in these components reflect dietary influences and phenotypic plasticity in response to environmental conditions.<sup>11</sup> Among these, crude protein and crude fat are particularly indicative of nutritional status and short-term energy allocation.<sup>12</sup> In this study, no significant differences were found in moisture and ash content among the experimental groups. However, significant variations were observed in crude protein and crude fat content. The corn group had the lowest crude protein content, differing significantly from other groups, whereas the control group showed the lowest crude fat content, differing significantly from the corn and formulated feed groups. These disparities are primarily attributed to the nutritional profiles of the respective diets.

The lower crude protein content in the corn group is likely associated with the suboptimal nutritional quality of its diet. Corn, as a sole energy source, lacks sufficient protein and essential amino acids, limiting muscle protein deposition in crayfish.<sup>13</sup> Both the soybean and formulated feed groups had significantly higher muscle crude protein content than the corn group, reflecting their protein-rich diets. Soybean, a high-quality plant protein, supplies essential amino acids for growth, whereas formulated feed enhances protein utilization via a balanced nutrient profile.<sup>6</sup> The formulated feed group also had higher crude fat content than the control group, suggesting the diet met energy demands for lipid retention. As reported by Dong et al.,<sup>14</sup> farmed crayfish typically have lower moisture content than wild-caught individuals, aligning with this study's results. Wild crayfish, facing limited food resources, retain

higher muscle moisture, whereas farming conditions with abundant feed may reduce moisture content.

Gumminess and chewiness are key textural parameters for evaluating the organoleptic quality of aquatic products. Gumminess reflects the adhesive properties during mastication, primarily determined by myofibrillar proteins and connective tissue components, while chewiness represents the work required for mastication, influenced by myofiber organization and connective tissue distribution.<sup>15</sup> In this experiment, the soybean group exhibited significantly greater gumminess and chewiness than other groups, suggesting enhanced connective tissue formation and myofibrillar protein cross-linking, leading to improved textural resilience.<sup>16</sup> These results corroborate Liu et al.,<sup>17</sup> where soybean-based high-protein feeds promoted myofiber hyperplasia, reduced fiber diameter, and collagen deposition, thereby improving muscle structural integrity.

Blood biochemical parameters serve as sensitive indicators of metabolic status and organ function, reflecting the physiological and nutritional status of crustaceans.<sup>10</sup> These parameters are modulated by multiple biotic and abiotic factors, including ontogenetic stage, husbandry practices, and environmental variables (temperature, salinity, photoperiod), as well as dietary formulation.<sup>18</sup> AST and ALT are hepatopancreas-specific enzymes involved in amino acid metabolism and serving as indicators of hepatopancreatic function. In this study, the absence of significant inter-group differences in AST and ALT activities suggests the feeding regimes maintained normal hepatopancreatic function in crayfish. AKP, a biomarker for exoskeletal formation and hepatobiliary function, normally exhibits elevated levels during larval and juvenile stages.<sup>19</sup> Similarly, the consistent AKP and Glu levels across groups indicate the feeding regimes preserved normal glucose homeostasis and exoskeletal mineralization processes.

TP (total protein) serves as a key biomarker of hepatopancreatic function and immune status, consisting of ALB (albumin) and GLB (globulin) fractions. ALB contributes to nutrient transport and hemolymph osmotic regulation, whereas GLB contains pathogen-recognition proteins and antimicrobial factors essential for innate immunity.<sup>20</sup> In this study, the formulated feed group exhibited significantly higher GLB levels compared to the con-

control group, with the differences in TP largely attributed to elevated GLB concentrations. Elevated GLB levels correlate with strengthened humoral immune capacity against opportunistic pathogens. The higher GLB levels in the formulated feed group may be associated with the elevated protein content in the diet, which supports protein synthesis and immune function.<sup>21</sup> Luo et al.<sup>22</sup> demonstrated that TP elevation reflects optimal dietary amino acid profiles, heightened metabolic turnover, and upregulated biosynthetic pathways. In this study, crayfish in the 1<sup>#</sup>, 1<sup>^</sup>, and 3<sup>#</sup> experimental groups received supplementary protein feed in addition to aquatic plants, resulting in more comprehensive nutrient intake compared to the control group. Consequently, these groups demonstrated significantly higher TP levels, further supporting the role of protein-rich diets in promoting growth and immune function in crayfish.

## CONCLUSION

In summary, the results of this study demonstrate the following key findings: (1) From the perspective of growth performance and nutritional composition, the cultivation mode combining formulated feed with *E. nuttallii* yielded the most favorable growth traits in crayfish; (2) In terms of muscle texture and sensory quality, the soybean-supplemented diet in conjunction with *E. nuttallii* produced superior outcomes; (3) With regard to serum biochemical parameters, the inclusion of *E. nuttallii* in the cultivation environment, supplemented with protein-rich feeds, significantly enhanced the overall farming efficacy of crayfish, with the formulated feed group exhibiting the most pronounced benefits.

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## AUTHORS' CONTRIBUTION

YYZ and GHM conceived and designed the research. GHM, LPS, SQM, YHZ and BLW conducted the experiment. PX, JW, XCZ, BL, SLX and WXZ analyzed the data. GHM wrote the paper. YYZ revised the paper.

## COMPETING INTEREST

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

## ETHICAL CONDUCT APPROVAL

All procedures were carried out according to the Institutional Animal Care and Use Committee Guide in Freshwater Fisheries Research Institute of Shandong Province.

## INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

## DATA AVAILABILITY STATEMENT

All data generated or used during the study appear in the submitted article.

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