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Research Article

Tragacanth Gum and Linseed Gum as Adhesives Improved the Survival, Digestive Function, Antioxidant Enzyme Activities, and Immunity in Large Yellow Croaker (*Larimichthys crocea*) Larvae

Jiahui Liu,[1] Yuntao Wang,[1] Jianmin Zhang,[1] Chenxiang Zhang,[1] Chuanwei Yao,[1] Wenxuan Xu,[1] Xiang Xu,[1] Kangsen Mai,[1,2] and Qinghui Ai[1,2]

[1]*Key Laboratory of Aquaculture Nutrition and Feed (Ministry of Agriculture and Rural Affairs), Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, China*
[2]*Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, 1 Wenhai Road, Qingdao, Shandong 266237, China*

Correspondence should be addressed to Qinghui Ai; qhai@ouc.edu.cn

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A 30-day feeding experiment was designed to investigate the effects of different adhesives on survival, growth performance, digestive enzyme activities, antioxidant capacity, and inflammation response of large yellow croaker (*Larimichthys crocea*) larvae (initial weight 8.40 ± 0.18 mg). Four iso-nitrogenous (55% crude protein) and iso-lipidic (22% crude lipid) microbound diets (MBD) were formulated to contain 2% sodium alginate (SA), 2% tragacanth gum (TG), 2% linseed gum (LG), and 2% poloxamer (PX), respectively. Results showed that larvae fed with TG and LG diets had significantly higher survival rate than the larvae fed with SA and PX ($P < 0.05$). Larvae fed with the PX diet had lower final weight and specific growth rate than the other groups ($P < 0.05$). Larvae fed with TG diet had significant higher activities of lipase in pancreatic segments and trypsin in intestinal segments than the larvae fed with the PX ($P < 0.05$). Meanwhile, the lipase activities of larvae fed with LG was significantly higher in intestinal segments than those fed with SA and PX diets ($P < 0.05$). Larvae fed with TG and LG diets had significantly higher activities of AKP in brush border membranes than larvae fed with SA and PX diets ($P < 0.05$). Moreover, the mRNA expression of pcna and zo-1 increased, respectively, in larvae fed TG and LG diets compared with the other groups ($P < 0.05$). Larvae fed with LG and TG diets had significantly higher activities of SOD and T-AOC than those fed with PX diet ($P < 0.05$). The content of GSH increased significantly in larvae fed with SA, TG, and LG diets compared with PX treatment ($P < 0.05$). The activities of iNOS significantly increased in larvae fed with TG and LG diets compared to other groups ($P < 0.05$). Meanwhile, the mRNA expression of il-10 significantly increased in TG and LG groups ($P < 0.05$). In conclusion, results of the study demonstrated that TG and LG can be used as feed binders for microdiets with positive effects on survival, activities of digestive enzymes, antioxidant capacity, and inflammatory response. However, PX inhibited the survival and growth, and further study is needed to determine the suitable usage and dosage.

1. Introduction

During the aquatic fry feeding stage, the requirements of food are very strict [1]. Traditionally, larvae of fish are fed with live feeds while they are expensive, fluctuating, and infectant [2]. Because of the high standards of feed of larvae, microbound diet (MBD), which has balanced nutrition and stable source of raw materials, is valued by fishery industry.

The application of different adhesives in MBD directly affects the dissolution of nutrients, palatability of feed, and nutrient absorption [3]. In the previous study, white sturgeon (*Acipenser transmontanus*) larvae had difficulty in digesting carrageenan substrates, which limits the

assimilation of nutrients [4]. Red Sea Urchin (*Pseudocentrotus depressus*) fed with casein and sodium alginate diets showed a higher growth rate and lower feed efficiency than those fed with casein and curdlan [5]. Agar-bound microparticulate diet was proved as great potential replacements to diatoms as alternative food for tropical abalone (*Haliotis asinina*) postlarvae in scale culture [6]. Therefore, suitable binder is significant to improve the quality of pellet and increase the breeding efficiency of aquatic animals.

Sodium alginate (SA), which is a by-product of kelp processing, is widely used in experimental feeds for excellent adhesion while it is expensive. Meanwhile, tragacanth gum (TG), which is extracted from astragalus (*Astragalus membranaceus*), is a multifunctional gum with unique thickening, emulsifying, and physiologically activities on its application [7, 8]. Linseed gum (LG) is made from linseed (*Linum usitatissimum* L.) or seed skin by extraction. LG has high viscidity and its main component is polysaccharide, which has effect of antioxidant, antiobesity, and regulating the intestinal flora [9, 10]. As a new kind of macromolecular nonionic surfactant with viscosity and no physiological activities, poloxamer (PX) can be used as polymer gelling agent for drug delivery [11]. In the field of aquaculture, SA is widely used as adhesives. However, TG, LG, and PX could be some promising adhesives and few studies were reported in fish larvae.

Large yellow croaker is widely farmed in China for its delicious taste and great economic value [12]. SA has been widely used as a binder in MBD for large yellow croaker. To date, no studies have been reported on TG, LG, and PX to evaluate its potential value as adhesives in microdiet. Therefore, the study was designed to investigate the effects of different adhesives on survival, growth performance, activities of digestive enzymes, antioxidant capacity, and inflammatory response of large yellow croaker larvae.

2. Materials and Methods

2.1. Diets Formulation. Four iso-nitrogenous (55% crude protein) and iso-lipidic (22% crude lipid) MBD were formulated to contain 2% SA (control group), 2% TG, 2% LG, and 2% PX, respectively (Table 1).

According to the feed formula, white fish meal, krill meal, squid meal, choline chloride, and yeast extract were mixed and ground to get premix 1. Then, different adhesives were proportionally dissolved in water and blended with α-starch, vitamin premix, mineral premix, monocalcium phosphate, ascorbyl polyphosphate, mould inhibitor, antioxidant, and soybean lecithin to get premix 2. At last, fish oil was mixed with premix 1 and premix 2 and granulated by microbonding technology. The process index was the mixing and stirring temperature was 26~35°C, the mixing and stirring rate was 300~500 rpm/min, the rotating speed of the axial single-screw spherical extruder was 50~90 rpm/min, and the rotating speed of the rounder was 1000~1500 rpm/min. The particle size of 0.2 mm microgranular feed was made after drying with hot air at 50~60°C for 30~45 min. All preparation was finished at Nankou base of Chinese Academy of Agricultural Science (Beijing, China).

Table 1: Formulation and proximate analysis of the experimental diets (% dry matter).

Ingredient% dry diet	Experiment diets			
	SA	TG	LG	PX
White fish meal[1]	35.00	35.00	35.00	35.00
Krill meal[1]	27.00	27.00	27.00	27.00
Squid meal[1]	10.00	10.00	10.00	10.00
Yeast extract[1]	3.50	3.50	3.50	3.50
α-starch	6.00	6.00	6.00	6.00
Vitamin premix[2]	1.50	1.50	1.50	1.50
Mineral premix[2]	1.00	1.00	1.00	1.00
Monocalcium phosphate	2.00	2.00	2.00	2.00
Ascorbyl polyphosphate	0.20	0.20	0.20	0.20
Mould inhibitor	0.05	0.05	0.05	0.05
Antioxidant	0.05	0.05	0.05	0.05
Choline chloride	0.20	0.20	0.20	0.20
Fish oil	6.50	6.50	6.50	6.50
Soybean lecithin[1]	5.00	5.00	5.00	5.00
Sodium alginate	2.00	0.00	0.00	0.00
Tragacanth gum	0.00	2.00	0.00	0.00
Linseed gum	0.00	0.00	2.00	0.00
Poloxamer	0.00	0.00	0.00	2.00
Analyzed nutrients composition (dry matter basis)				
Crude protein (%)	55.30	55.68	54.72	55.22
Crude lipid (%)	22.21	21.89	21.92	22.52

[1]Raw material was purchased from Qingdao Seven Good Biological Technology Co., Ltd., in Shandong, China; elementary composition (dry matter) referred to a previous study [13]. [2]Vitamin premix (IU or g·kg^{-1}), Mineral premix (IU or g·kg^{-1}): elementary composition referred to a previous study [13].

Tragacanth gum, linseed gum, sodium alginate, and poloxamer were purchased from Zhejiang Yinuo Bio-Engineering Institute, China.

Tragacanth gum and linseed gum was prepared by the following methods: the oil in astragalus and flaxseed was removed, and the powder was obtained after crushing. The powder was mixed with water and stirred for 4 hours. Finally, the powder of the gum was obtained after filtration and distillation. The above method was repeated three times.

2.2. Experimental Procedure and Fish Rearing Conditions. Large yellow croaker larvae were raised in the Institute of Marine and Fisheries Research of Ningbo, China. Large yellow croaker larvae were fed with rotifers (*Brachionus plicatilis*) (0.5×10^4~1.5×10^4 individual L^{-1}) from 3 to 7 days after hatching (DAH), brine shrimp (*Artemia nauplii*) (1.0×10^3~1.5×10^3 individual L^{-1}) from 5 to 10 DAH, copepods (*Calanus sinicus*) from 8 to 14 DAH and the same MD from 12 to 14 DAH. Then, the large yellow croaker larvae (15 DAH, average body weight 8.40 ± 0.18 mg) were randomly divided into 12 blue plastic tanks (water volume 200 L, 3000 larvae per tank) and completely fed with experimental feed. The method was referred to a previous study [13].

During the feeding experiment (30 days), larvae were fed eight times a day (7:00, 9:30, 12:00, 14:30, 17:00, 19:30, 22:00, and 24:00). The aquatic water was renewed about 100~200%

daily and the parameters were strictly controlled (pH value: 7.8~8.2, salinity: 21‰~24‰, and temperature: 23~26°C) [14].

2.3. Sample. At the beginning of the experiment, 50 larvae of 15 DAH were randomly collected to measure initial body length (IBL) and initial body weight (IBW). At the end of the feeding trial, larvae were fasted for 24 h before sample collection. 80 larvae were randomly selected to separated visceral mass (VM, containing liver, intestine, heart, pancreas, and spleen) and separate pancreatic segments (PS) and intestinal segments (IS) under an anatomical microscope at 0°C. Samples were quickly placed in 2.0 mL RNase-free cryogenic vials and stored in a −80°C refrigerator for measuring enzyme activities and gene expression analysis. The remaining larvae were gathered form each tank and stored at −20°C for body composition assay [15].

2.4. Analytical Methods

2.4.1. Leaching Rate. After drying of feed, three samples (10 g, one control group and two test groups) were prepared. Two test groups were placed in a wire screen in a container (5.5 cm deep) with seawater (15 gL^{-1} salinity, pH 8.0) for a parallel test. After 30, 60, 90, and 120 min, the feed was lifted up and down three times from the bottom to the surface of water and dried until constant weight. All determinations were made in triplicate.

2.4.2. Component Analysis. Samples of MBD and larvae were dried at 105°C until constant weight to determine crude protein (we used Kjeldahl method and multiply nitrogen by 6.25, Kjeltec TM 8400, FOSS, Tecator, Sweden) and crude lipid (we used Soxhlet method, B-801, Switzerland). Each sample was analyzed three times.

2.4.3. Enzyme Activities Assay. Samples of 0.1 g IS and PS were ground with 1 ml phosphate-buffered saline (4°C, pH = 7.4) and centrifuged at 3000 g for 10 min to obtain supernatant for the following assays. The supernatant was determined for α-amylase, trypsin, lipase, and alkaline phosphatase activity (AKP) according to commercial test kits. Following the method of Crane [16], the purified brush border membranes (BBM) of the IS were extracted for the measurement of AKP. The activities of leucine-aminopeptidase (LAP) were assayed in the BBM according to published paper [17].

Samples of 0.1 g VM were ground with 1 ml saline and centrifuged at 3000 g for 10 min to obtain the supernatant. The activities of superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (T-AOC), lysozyme (LZM), total nitric oxide synthase (TNOS), inducible nitric oxide synthase (iNOS), and constitutive citric oxide synthase (cNOS) were determined by commercial test kits. The content of malondialdehyde (MDA), triglyceride, and glutathione (GSH) were also determined by commercial test kits. Commercial test kits were purchased from Nanjing Jiancheng Bioengineering Institute, China.

2.4.4. RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Samples of 0.01 g VM were added into 1 ml Trizol (Takara, Japan) reagent for grounding to get homogenate. The total RNA was extracted and purified from the homogenate and detected for integrity and quality (using 1.2% denatured agarose gel electrophoresis). Then, the concentration of RNA was assessed by a Nano Drop®2000 spectrophotometer (Thermo Fisher Scientific, USA). The total RNA was reversely transcribed to cDNA by Prime Script-RT reagent Kit (Takara, Japan). A quantitative thermal cycler (CFX96 TM Real-Time System, BIO-RAD, USA) was applied to carry out real-time quantitative polymerase chain reaction [13, 18]. PCR primer sequences were directly synthesized in this study (Table 2).

2.5. Calculation and Statistical Methods

2.5.1. Growth Performance

$$\text{Survival rate (SR) (\%)} = \frac{N_t \times 100}{N_0},$$

$$\text{Specific growth } \left(\text{SGR, \% day}^{-1}\right) = \frac{\left(\text{In } W_t - \text{In } W_0\right) \times 100}{D}, \quad (1)$$

where N_t represents total number of larvae at the ending of experiment, N_0 represents total number of larvae at the beginning of experiment, W_t represents final body weight of larvae, W_0 represents initial body weight of larvae, and D represents total number of experimental days.

2.5.2. Leaching Rate. The stability of microdiets is expressed by the following leaching rate:

$$C = \frac{(m_0 - m) \times 100}{m_0}, \quad (2)$$

where C represents leaching rate; %. m_0 represents the weight of control group after drying, unit is g; and M represents the weight of test group after drying, unit is g.

2.5.3. Statistical Analyses. All data in this study were analyzed by SPSS statistics for mac V26.0 (IBM, America) by one-way analysis of variance (ANOVA), and then determined by Tukey's range test. The significance level was determined as $P < 0.05$ and results were exhibited as mean ± S.D. (Standard Deviation).

3. Results

3.1. Leaching Rate. The leaching rate of different groups increased over time and LG group retained more nutrients than other groups after immersion in water for 90 min ($P < 0.05$) (Figure 1). There was no significant difference among SA, TG, and PX groups ($P > 0.05$) (Figure 1).

Table 2: PCR primer sequences used in this study.

Target gene	Forward primers (5′-3′)	Reverse primers (5′-3′)	Accession number
zo-1	TGTCAAGTCCCGCAAAAATG	CAACTTGCCCTTTGACCTCT	XM019260744
zo-2	ACCCGACCTGTTTGTTATTG	ATGCCGTGCTTGCTGTC	XM027276911
occludin	AGGCTACGGCAACAGTTATG	GTGGGTCCACAAAGCAGTAA	XM010740442
pcna	GAGAGACAAGTGAGAGTTACCG	CTCTTTGTCTACATTGCTGGTCT	XM010734227
odc	GAGCCAGGTCGCTTCTATG	CCGTGGTCCCTTCGTCT	XM010736389
claudin	ACCTCCGCCATCAAGCA	TGGGACAAAGAGGCCACATC	XM010749420
tnf-α	ACACCTCTCAGCCACAGGAT	CCGTGTCCCACTCCATAGTT	NM001303385
il-1β	CATAGGGATGGGGACAACGA	AGGGGACGGACACAAGGGTA	XM010736551
il-6	CGACACACCCACTATTTACAAC	TCCCATTTTCTGAACTGCCTCT	XM010734753
il-8	AATCTTCGTCGCCTCCATTGT	GAGGGATGATCTCCACCTTCG	XM010737667.3
il-10	AGTCGGTTACTTTCTGTGGTG	TGTATGACGCAATATGGTCTG	XM010738826
β-actin	GACCTGACAGACTACCTCATG	AGTTGAAGGTGGTCTCGTGGA	GU584189

il-1β, interleukin-1β; tnf-α, tumor necrosis factor-α; il-6, interleukin-6;il-8, interleukin-8;il-10, interleukin-10;zo-1, tight junction protein-1;zo-2, tight junction protein-2; pcna, proliferating cell nuclear antigen; odc, ornithine decarboxylase.

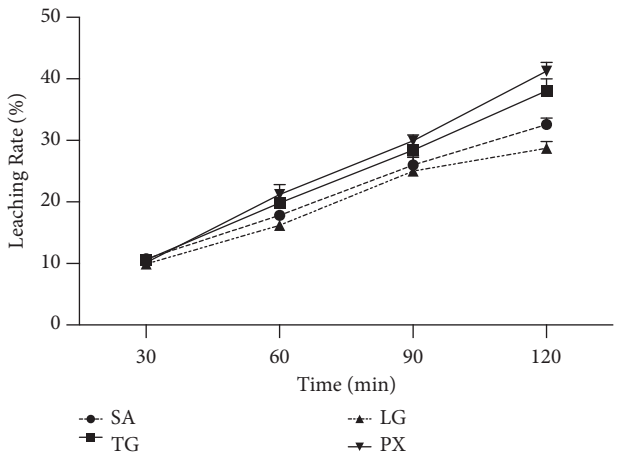

Figure 1: Leaching rate of total nutrients from microdiets groups. (Means ± S.D., $n = 3$).

3.2. Growth Performance, Survival Rate, and Body Composition. The SR of larvae fed with TG and LG diets was significantly higher than dietary SA and PX ($P < 0.05$) (Table 3). Larvae fed with PX diet had significantly lower FBW and SGR than those fed with other diets ($P < 0.05$) (Table 3). There was no statistical difference of crude protein, crude lipid, and moisture among different treatments ($P > 0.05$) (Table 4).

3.3. Digestive Enzyme Activities. The activities of trypsin in IS of larvae fed with SA, TG, and LG was significantly higher than PX treatments ($P < 0.05$) (Table 5). The activities of α-Amylase (PS) and lipase (PS) of larvae fed with TG diet significantly increased compared to those fed with PX diet ($P < 0.05$) (Table 5). Meanwhile, the larvae fed with LG diet had significantly higher activities of lipase (IS) than those fed with SA and PX diets ($P < 0.05$) (Table 5). Larvae fed with TG and LG diets had significantly higher activities of AKP than other groups in BBM ($P < 0.05$) (Table 5). Meanwhile, larvae fed with the TG and LG diets significantly increased the activities of LAP in BBM compared with the PX diet ($P < 0.05$) (Table 5).

3.4. Expression of Intestinal Development Related Genes. Larvae fed LG diet had significantly higher mRNA expression of zo-1 in VM than larvae fed other diets ($P < 0.05$) (Figure 2). Larvae fed TG diet had significantly higher mRNA expression of pcna than larvae fed other diets ($P < 0.05$) (Figure 2). Moreover, larvae fed with TG and LG diets significantly increased the mRNA expression of occludin compared with the other groups ($P < 0.05$) (Figure 2). There was no significant difference in the expression of zo-2, odc, and claudin ($P > 0.05$) (Figure 2).

3.5. Activities of Antioxidant Enzymes. Larvae fed LG and TG diets had significantly higher activities of SOD and T-AOC than those fed with PX diet ($P < 0.05$) (Table 6). However, there was no significant difference between different groups in the activities of CAT ($P > 0.05$) (Table 6). The content of MDA decreased significantly in larvae fed with SA and TG diets compared to the other groups ($P < 0.05$) (Table 6). The content of GSH increased significantly in larvae fed with SA, TG, and LG diets compared with PX diet ($P < 0.05$) (Table 6). The content of triglyceride was significantly lower in larvae fed with SA, TG, and LG diets than larvae fed the PX diet ($P < 0.05$) (Table 6).

3.6. Inflammatory Response. No significant differences were observed in the activities of LZM among different dietary treatments ($P < 0.05$) (Table 7). Larvae fed with the LG diet had significantly higher activities than those fed with SA and PX diets ($P < 0.05$) (Table 7). Meanwhile, the activities of iNOS significantly increased in larvae fed with the TG and LG diets compared to the other groups ($P < 0.05$) (Table 7).

Larvae fed with TG and LG diets had significantly lower tnf-α transcriptional levels than the other groups ($P < 0.05$) (Figure 3). Moreover, the mRNA expression of il-10 significantly increased with the larvae fed with TG and LG diets ($P < 0.05$) (Figure 3).

4. Discussion

MBD is widely used in aquaculture for its high production efficiency and simple process. However, the weak binding of adhesives could lead to nutrition loss and deterioration of

Table 3: Effects of different adhesives on growth performance and survival rate (Means ± S.D., $n = 3$)[a].

Parameters	Experiment diets				P-value
	SA	TG	LG	PX	
IBW[1] (mg)	8.40 ± 0.18	8.40 ± 0.18	8.40 ± 0.18	8.40 ± 0.18	—
FBW[1] (mg)	172.67 ± 2.91[a]	177.11 ± 2.03[a]	179.11 ± 3.80[a]	160.22 ± 3.23[b]	<0.001
SGR[1] (%/day)	9.81 ± 0.06[a]	9.90 ± 0.04[a]	9.94 ± 0.07[a]	9.56 ± 0.07[b]	<0.001
IBL[1] (mm)	9.09 ± 0.19	9.09 ± 0.19	9.09 ± 0.19	9.09 ± 0.19	—
FBL[1] (mm)	20.73 ± 0.78	21.01 ± 0.18	20.74 ± 0.24	20.49 ± 0.13	0.543
SR[1] (%)	17.54 ± 0.64[b]	20.59 ± 1.02[a]	19.96 ± 1.05[a]	16.23 ± 0.38[b]	0.001

[a]Through Tukey's test, data with the same superscript letter in the same row have no significant difference ($P > 0.05$). [1]IBW: initial body weight; FBW: final body weight; SGR: specific growth rate; IBL: initial body length; FBL: final body length; SR: survival rate.

Table 4: Effects of different adhesives on body composition (Means ± S.D., $n = 3$)[a].

Parameters	Experiment diets				P-value
	SA	TG	LG	PX	
Crude protein (%)	55.30 ± 1.42	55.68 ± 0.41	54.72 ± 2.32	55.22 ± 1.45	0.899
Crude lipid (%)	22.21 ± 1.17	21.89 ± 0.94	21.92 ± 0.93	22.52 ± 0.42	0.816
Moisture (%)	86.55 ± 1.41	86.69 ± 1.16	86.21 ± 1.02	86.98 ± 1.19	0.885

[a]Through Tukey's test, data with the same superscript letter in the same row have no significant difference ($P > 0.05$).

Table 5: Effects of different adhesives on the activities of digestive enzymes (Means ± S.D., $n = 3$)[a].

Parameters		Experiment diets				P-value
		SA	TG	LG	PX	
α-amylase (U/mg protein)	PS[1]	0.15 ± 0.01[ab]	0.18 ± 0.01[a]	0.17 ± 0.01[ab]	0.14 ± 0.03[b]	0.038
	IS[1]	0.20 ± 0.02	0.24 ± 0.01	0.22 ± 0.03	0.20 ± 0.02	0.127
Trypsin (U/mg protein)	PS[1]	3.97 ± 0.09	3.77 ± 0.12	3.91 ± 0.38	3.66 ± 0.34	0.489
	IS[1]	4.84 ± 0.21[a]	4.92 ± 0.53[a]	4.85 ± 0.66[a]	3.53 ± 0.12[b]	0.011
Lipase (U/g protein)	PS[1]	0.60 ± 0.09[ab]	0.81 ± 0.07[a]	0.79 ± 0.15[ab]	0.54 ± 0.07[b]	0.023
	IS[1]	0.73 ± 0.03[bc]	0.86 ± 0.05[ab]	0.92 ± 0.08[a]	0.71 ± 0.06[c]	0.005
AKP[2] (mU/mg·protein)	BBM[1]	64.14 ± 2.20[b]	83.50 ± 4.00[a]	79.44 ± 7.92[a]	57.48 ± 5.21[b]	0.001
LAP[2] (mU/mg·protein)	BBM[1]	6.16 ± 0.83[ab]	7.25 ± 0.79[a]	7.42 ± 0.93[a]	4.31 ± 0.26[b]	0.003

[a]Through Tukey's test, data with the same superscript letter in the same row have no significant difference ($P > 0.05$). [1]PS: pancreatic segments; IS: intestinal segments; BBM: brush border membranes. [2]AKP: alkaline-phosphatase; LAP: leucine-aminopeptidase.

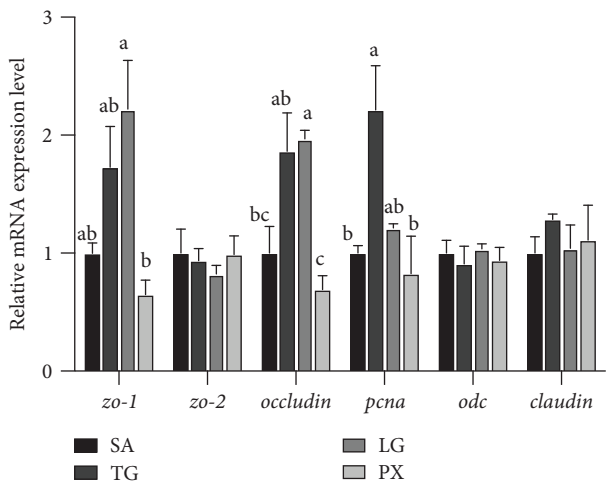

Figure 2: Effects of different adhesives on zo-1, zo-2, occludin, pcna, odc, and claudin mRNA expression in a visceral mass. Vertical bars represent standard errors. There was no significant difference in bars bearing the same letters ($P > 0.05$, Tukey's test). zo-1, tight junction protein-1;zo-2, tight junction protein-2; pcna, proliferating cell nuclear antigen; and odc, ornithine decarboxylase.

water quality [19]. In this study, diets that used LG as a binder showed higher nutrient retention after immersion in water for 90 min. In order to prolong the time of stabilization in water, LG and PX as binders can be used in combination with other binders [20, 21].

In this study, results demonstrated that different adhesives had specific effects on survival and growth performance in large yellow croaker larvae. Previous study on astragalus polysaccharide as the main component of TG proved that *Astragalus* polysaccharide could raise the SR and growth performance of aquatic animals such as crucian carp (*Carassius auratus*) and large yellow croaker [22, 23]. It is important that larvae fed with LG and TG diets significantly improved the SR while having no difference in growth performance, which was different from the results of previous experiments. Meanwhile, the sustained-release of PX may hinder the nutrient intake of larvae fed PX, which resulted in poor growth performance [24].

The improvement of digestive enzyme activities represents the intestinal development and good nutritional status of aquaculture animals [25, 26]. Results showed larvae fed with the SA, TG, and LG diets had significantly higher

Table 6: Effects of different adhesives on the activities of antioxidant (Means ± S.D., $n = 3$)[a].

Parameters	Experiment diets				P-value
	SA	TG	LG	PX	
SOD[1] (U/mg protein)	7.10 ± 0.76^{ab}	9.83 ± 1.16^{a}	10.49 ± 2.33^{a}	6.06 ± 0.7^{b}	0.013
T-AOC[1] (Trolox/g protein)	0.11 ± 0.02^{ab}	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.10 ± 0.01^{b}	0.030
CAT[1] (U/mg protein)	18.51 ± 2.52	18.20 ± 1.84	20.20 ± 2.25	17.24 ± 0.75	0.374
MDA[1] (nmol/mg protein)	10.88 ± 2.40^{a}	9.28 ± 4.05^{a}	11.68 ± 1.53^{ab}	18.03 ± 2.13^{b}	0.018
GSH[1] (nmol/mg protein)	85.56 ± 11.91^{b}	104.13 ± 13.67^{ab}	125.45 ± 10.92^{a}	69.14 ± 9.79^{c}	0.002
Triglyceride (mmol/mg protein)	0.40 ± 0.05^{a}	0.36 ± 0.05^{a}	0.34 ± 0.03^{a}	0.52 ± 0.03^{b}	0.004

[a]Through Tukey's test, data with the same superscript letter in the same row have no significant difference ($P > 0.05$). [1]SOD: superoxide dismutase; T-AOC: total antioxidant capacity; CAT: catalase; MDA: malondialdehyde; GSH: glutathione.

Table 7: Effects of chitosan-coated diets on the activities of immunity (Means ± S.D., $n = 3$)[a].

Parameters	Experiment diets				P-value
	SA	TG	LG	PX	
LZM[1] (U/mg protein)	111.26 ± 10.60	108.61 ± 11.48	114.20 ± 4.36	101.25 ± 6.20	0.362
TNOS[1] (mU/mg protein)	1.85 ± 0.10^{b}	2.19 ± 0.16^{ab}	2.37 ± 0.30^{a}	1.66 ± 0.05^{c}	0.004
iNOS[1] (mU/mg protein)	1.40 ± 0.16^{b}	1.82 ± 0.16^{a}	1.81 ± 0.17^{a}	1.26 ± 0.12^{b}	0.004
cNOS[1] (mU/mg protein)	0.45 ± 0.08	0.36 ± 0.05	0.57 ± 0.14	0.39 ± 0.08	0.107

[a]Through Tukey's test, data with the same superscript letter in the same row have no significant difference ($P > 0.05$). [1]LZM: lysozyme; TNOS: total nitric oxide synthase; iNOS: inducible nitric oxide synthase; cNOS: constitutive citric oxide synthase.

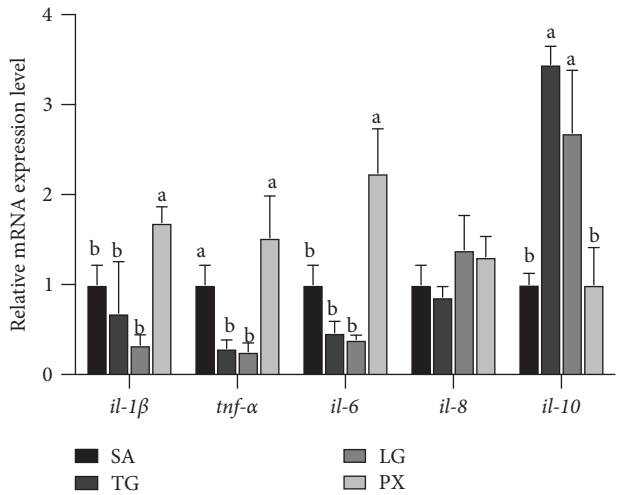

Figure 3: Effects of dietary adhesives on il-1β, tnf-α, il-6, il-8, and il-10 mRNA expression in a visceral mass. Vertical bars represent standard errors. There was no significant difference in bars bearing the same letters ($P > 0.05$, Tukey's test). il-1β, interleukin-1β; tnf-α, tumor necrosis factor-α; il-6, interleukin-6;il-8, interleukin-8; and il-10, interleukin-10).

activities of trypsin than larvae fed with the PX diet. This is consistent with the results of growth performance of larvae. Previous studies confirmed that feeding sodium alginate, astragalus, and linseed could improve the activities of digestive enzymes in Asian seabass (*Lates calcarifer*) and silver catfish (*Rhamdia quelen*) [27–29]. Meanwhile, AKP and LAP were considered as the important indicator of digestive function, which is located in brush border membranes of fish foregut epithelial cells [30, 31]. In this study, TG and LG addition improved the activities of AKP of large yellow croaker larvae. Moreover, addition of SA, TG, and LG could positively influence the expression of genes related to proliferation and development of larvae intestine. Larvae fed with TG and LG diets, respectively, increased the mRNA expression of pcna and zo-1 compared with the other groups. zo-1 and pcna are essential for intestinal barrier and hemocytes proliferation [32, 33]. Meanwhile, addition of TG and LG significantly improved the mRNA expression of occludin, which can enhance barrier function [33, 34]. The reason why dietary TG and LG were beneficial to digestive enzymes might attribute to the development of the digestive tract [35, 36].

Marine fish are very vulnerable in the early stage, and supplementation with antioxidants can strengthen the antioxidant system to resist oxidative injury such as DNA damage and lipid peroxidation [37, 38]. SOD was involved in early-stage antioxidant defense, which reflected the antioxidant status of animals [39]. Results showed that larvae fed TG and LG diets had significantly higher activities of SOD than the other treatments. The result was similar to the study on white shrimp (*Litopenaeus vannamei*) and tilapia (*Oreochromis niloticus*) [40, 41]. Meanwhile, dietary TG and LG significantly improved the content of GSH, which worked in conjunction with SOD to eliminate potential toxic oxidation products [42]. Results showed that larvae fed with PX diet had significantly higher content of MDA and triglyceride. The result was similar to the study on rabbit that PX could induce a dose-dependent hyperlipidemia [43]. To sum up, LG and TG as natural antioxidant foods could improve antioxidant enzymes of large yellow croaker larvae to cope with oxidative stress.

LZM and TNOS are the component of fish innate immune system that improve disease resistance at the early stage of larvae [44, 45]. Larvae fed with SA, TG, and LG diets significantly increased the activities of TNOS compared with those fed PX diet. Meanwhile, TG and LG diets significantly

improved the activities of cNOS, which was involved in helping in defense and ion regulation and sensory functions, increasing the adaptability and survival of the larvae in harsh condition [45]. Inflammation was considered as an alarming signal for the progress of varied biological complications [46]. This study selected 5 factors (il-1β, tnf-α, il-6, il-8, and il-10) to investigate the effects of different adhesives on inflammatory response. Larvae fed with TG and LG diets had significantly lower mRNA expression of tnf-α which is an important proinflammatory factor. Moreover, il-10 is an anti-inflammatory cytokine produced mainly by macrophages and plays a role in counteracting and controlling excessive inflammatory response [47–49]. In the present study, the mRNA expression of *il-10* significantly increased with the larvae fed with TG and LG diets. The immunomodulatory effects of astragalus and linseed have been proved in Nile tilapia, barramundi (*Lates calcarifer*), silver catfish, and Chinese Holstein dairy cow [50–53]. These results indicated that supplementation of TG and LG could reduce inflammation and further improve survival by enhancing the immune system.

5. Conclusion

In conclusion, results of the study illustrated that TG and LG can be used as feed binders for microdiets of large yellow croaker larvae with positive effects on survival, activities of digestive enzyme, antioxidant capacity, and inflammatory response. However, PX inhibited the digestion and growth of large yellow croaker larvae and further study is needed to determine the suitable usage and dosage.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

This study was carried out in strict with the recommendations in the Guide for the Use of Experimental Animals of the Ocean University of China.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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