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Effect of oligochitosan supplementation on growth and innate immunity of striped catfish (*Pangasianodon hypophthalmus)* at pond culture

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Abstract: Oligochitosan (COS) was prepared by gamma Co-60 irradiation of chitosan/H_2O_2 solutions and mixed with cornstarch to form the powder with content of 100 000 mg COS/kg. The striped catfish (*P. hypophthalmus***)** was fed with diets containing 100 mg COS/kg feed for 9 months at pond culture. The effects of COS supplementation on growth performance, feed conversion ratio (FCR) and survival rate in striped catfish were investigated. The results indicated that the growth performance and survival rate of striped catfish fed with 100 mg COS/kg were significantly improved and the FCR decreased. The average weight gain, survival rate and FCR of striped catfish fed with diets containing COS were 971 ± 85 gram, 83.19 ± 0.35 % and 1.477 ± 0.013 in comparison with 896 ± 78 gram, 78.43 ± 0.64 % and 1.578 ± 0.038 of control group, respectively. Thus, COS can be potentially utilized as immunostimulants and growth promoter for aquaculture.

Keywords: *Oligochitosan; Striped catfish; Immunostimulant; Survival rate.*

I. INTRODUCTION

The striped catfish (*Pangasianodon hypophthalmus)* is one of the most important commercial fish species in South-East Asia, particularly in Mekong Delta, of Vietnam due to a big profit from annual catfish export [1]. The rapid expansion of culture and high farming intensity under inappropriate control resulted in serious diseases for striped catfish [2]. Nowadays, the use of immunostimulants deriving from natural polysaccharides for increasing the non-specific immune response has received considerable attention and became an alternative method for the prevention and control of various diseases in aquaculture. Chitosan is commonly prepared by sodium hydroxide deacetylation of chitin from crab, shrimp shells and squid pens, and consists of glucosamine and N-acetyl glucosamine units linked by β(1–4) glycoside bonds [3]. Chitosan has many unique properties such as antimicrobial activity [4], antioxidant activity and antitumor activity [5]. These features, combined with the biocompatibility, biodegradability and non-toxicity implies that chitosan is an interesting polymer for several applications in medicine, cosmetic,

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biotechnology, food and agriculture [6]. In aquaculture, due to the ability to enhance the non-specific immunity and resistance against pathogenic infection, chitosan is utilized as an immunostimulant [7, 8]. Several feeding trials and in vitro tests have shown that chitosan is able to enhance the resistance of aquatic animals against infections as well as immune capacities such as phagocytosis, superoxide anion production and lysozyme activity in fishes [9-13]. Other reports have shown immune stimulatory activity of chitosan in range of fish including koi, (*Ciprinus carpio koi)* [9, 10], rainbow trout (*Oncorhynchus mykiss*) [11, 12], ovate pompano (*Trachinotus ovatus*) [13], kelp grouper (*Epinephelus bruneus*) [14], tilapia (*Oreochromis niloticus)* [15].

In spite of these advantages, chitosan also has several drawbacks including poor solubility under physiological functions [10]. To improve these poor physicochemical properties, oligochitosan (COS) has been prepared and used. COS has a higher activity and more physiological functions than chitosan due to their low molecular weight, good solubility and low viscosity [16]. COS was recently shown to have immune-enhancing characteristics and protect against pathogenic infections [18, 20]. In our previous study, the effects of COS supplementation on immune stimulation and growth performance in striped catfish were investigated at laboratory scale [17]. The results indicated that for striped catfish fed with COS, the growth performance was significantly improved with the decrease in mortality. Striped catfish fed with 100 mg COS/kg feed was the highest increase of weight gain (~26%) and decrease of mortality (38.73%) compared with control group. However, not much data are available on administering COS as immunostimulant to improve the growth performance and immune response of fish at large-scale. Therefore, the aim of the present study was to investigate the effect of COS prepared by gamma Co-60 on growth and innate immunity of striped catfish (*P. hypophthalmus)* at pond culture.

II. MATERIALS AND METHODS

A. Oligochitosan preparation

Chitosan from shrimp shell with molecular weight (Mw) of 91.7 kDa and deacetylation degree of 91.3% was purchased from Chitosan Co., Vung Tau province. Chitosan was swollen in solution hydrogen peroxide (H_2O_2) at 1% concentration with ratio 1:20 (w/v) for 24 h. Then, 2% (w/v) lactic acid solution was added into the mixture to dissolve completely chitosan. A required amount of 30% H_2O_2 was added together with water to prepare solutions with concentration of 5% chitosan (w/v) and 0.5% H_2O_2 (w/v). Then, the resulting solutions were irradiated under ambient temperature on gamma SVST Co-60/B irradiator at the VINAGAMMA Center with the absorbed dose up to 20 kGy and the dose rate of 1.13 kGy/h. The Mw of COS measured by gel permeation chromatography (GPC), LC 20AB, Shimadzu was of 5600 Da. Oligochitosan solution was mixed with corn starch at 100 000 mg/kg, and then dried and grinded by grinder machine to form powder (COS/CS).

B. Diet preparation

The ingredient basal diet (control) comprised 22÷30% crude protein, 6 ÷ 10% crude lipid, 10÷14% crude ash and 6-8% fiber. To prepare the experimental diet, the basal diet was mixed with COS/CS at 100 mg COS/kg. The ingredients of the experimental diet were well-mixed and extruded through a pelletizer machine at the feed factory in Long An province. Total required amount of feed for testing is about 600 tons.

C. Fish and experimental conditions

Fish

Eight hundred thousand striped catfishes (*Pangasianodon hypophthalmus*) of 44.4 ± 8.1 g in body weight were brought from National Breeding Centre for Southern Freshwater Aquaculture, Tien Giang city, Vietnam. They were allowed acclimatizing to pond conditions for 30 days prior to use in experiments. The fishes were divided randomly into 5 ponds. The basic physico-chemical water parameters such as dissolved oxygen, NH_3, pH and temperature were checked daily to maintain the optimal level. The water temperature was controlled within the range of 25 – 28 ^oC. During the acclimatization period, fishes were fed daily with the basal diet.

Experimental design

This study comprises 5 ponds. Pond 1, 2 and 3 the striped catfish were fed diets with 100 mg COS/kg feed. Pond 4 and 5 the striped catfish were fed diets with no COS supplementation. All experiments were designed as in table I:

Table I. Area surface water , number of fish per pond, density and average weight of fish of each experiments

	Pond				
	A1	A 2	A 3	A 4	A 5
Area surface water (m^2)	2400	2500	2800	2500	2260
Number of fish per pond	154800	152500	178000	162000	145600
Density (fish/m^2)	65	61	64	65	64
Average weight (g/a fish)	41.5 ± 8.3	38.4 ± 8.1	40.3 ± 7.9	51± 8.5	51± 8.4

In all experiments, fishes were fed at the rate 1-10% of the body weight, twice a day for 9 months.

Survival rate and growth performance

Every month, 30 fish were caught randomly to measure body weight in order to calculate weight gain and the growth rate. Mortality of fish in each pond was recorded daily up to 9 months. At the end termination of the experiment, the fishes were fasted for 24h before harvest. Total number was counted and mean body weight of fish was measured. Based on the weight of each fish and the number of striped catfishes, weight gain (WG), survival and feed conversion ratio (FCR) were calculated as follows:

WG = final weight (g) – initial weight (g)

Survival (%) = 100 × (final number of striped catfish ÷ initial number of striped catfish)

FCR = Feed given (dry weight) ÷ weight gain (wet gain)

Statistics analysis

All the results were statistically analyzed by analysis of variance (ANOVA; MSTAT C, version. 1.2, Ann Arbor, MI, USA, 1989). The means were compared using the least significant difference (LSD) at 0.05 probability level ($P < 0.05$).

III. RESULTS AND DISCUSSION

A. Growth performance and feed conversion ratio (FCR)

Several researches have showed relationship between immunostimulant and

growth performance. In our previous study at laboratory condition, stripped catfish were fed COS at 50, 100 and 200 mg/kg concentration for 45 days. The results showed that dietary supplementation of COS significantly enhanced the growth performance and 100 mg COS/kg feed was optimal concentration of COS as immunostimulant for striped catfish. Therefore, in this study 100 mg COS/kg feed was selected to evaluate the effect of COS supplementation on growth and innate immunity of striped catfish at pond culture. Table II presented the growth performance data for striped catfish after 9 months experiment. The results clearly showed that the final weight and weight gain of striped catfish fed with 100 mg COS/kg feed was higher than those of the control groups. The average weight gain of striped catfish fed with diets containing COS was 971± 85 g compare to 896 ± 78 g of control group. In addition, the FCR of striped catfish feeding by COS was lower than those of control. The average FCR reduced from 1.578 ± 0.038 for the control groups to 1.477 ± 0.013 for COS supplementation groups.

Table II: Growth parameters and FCR of the striped catfish fed with the basal diets and diets containing 100 mg COS/kg after 9 months of feeding trial (mean ± SD)

Treatment	Initial weight (g)	Final weight (g)	Weight gain (g)	FCR	Average Weight gain (g)	Average FCR
A1*	41.5 ± 8.3	995 ± 61	954± 54	1.464	971 ± 85[a]	1.477 ± 0.013[a]
A2*	38.4 ± 8.1	1026± 112	985± 108	1.476		
A3*	40.3 ± 7.9	1015± 95	974± 94	1.489		
A4**	51± 8.5	892± 78	841± 76	1.604	896 ± 78[b]	1.578 ± 0.038[b]
A5**	51± 8.4	900± 84	849± 81	1.551		

*** Control: without supplementation of COS; * Supplementation of 100 mg COS/kg. The mean values in a column with the same letter are not significantly different (P<0.05).*

The influences of dietary COS supplementation on growth have been investigated with several aquaculture species with varied results. According to Lin et al. [10] dietary COS supplementation at 4000 mg/kg feed enhanced the growth of Derbio pompano (*Trachinotus ovatus*). A similar result was also observed in the study of Lin et al. [13] dietary supplementation with COS at level 500 mg/kg also enhanced the growth of koi (*Cyprinus carpio koi*). To date, how the immunostiumulants work to enhance the growth is not clear yet. Lin et al. proposed one hypothesis that after administration, a local intestinal inflammatory response induces resistance against pathogens which otherwise would result in the decrease in weight gain [18].

B. Survival rate

Infectious disease and adverse environmental condition are major impediments to the development of aquaculture, which lead to production and economic loss. The use of natural immunostimulant in aquaculture has been considered as an environmentally friendly method because they are biocompatible, biodegradable and harmless for the environment and human health [10, 13].

Average survival rate of striped catfish fed with diets with and without 100 mg COS/kg feed were presented in table III. The results of the present study clearly showed that dietary COS enhanced survival rate of striped catfish**.** The average survival of striped catfish fed with 100 mg COS/kg feed in A1, A2 and A3 ponds for 9 months was 83.19 ± 0.35% compare to that of 78.43 ± 0.64% in control ponds. The increase in the survival rate of striped catfish fed with COS may be explained by the augmentation of non-specific immunity, leading to the enhancement of the fish health and improvement of fish resistance against unfavorable environmental conditions.

Table III. Survival rate of the striped catfish fed with the basal diets and diets containing 100 mg COS/kg after 9 months of feeding trial

Treatment	Initial number of fish	Final number of fish	Survival rate (%)	Average survival rate (%)
A1*	154800	128484	83.00	83.19 ± 0.35[a]
A2*	152500	127503	83.61	
A3*	178000	147740	83.00	
A4**	162000	126360	78.00	78.43 ± 0.64[b]
A5**	145600	114890	78.91	

*** Control: without supplementation of COS; * Supplementation of 100 mg COS/kg. The mean values in a column with the same letter are not significantly different (P<0.05).*

The effect of dietary COS on survival rate has been studied by several authors. According to Lin et al. [18], dietary of chitosan significantly enhanced the non-specific immunity of koi through the decrease of average mortality and improvement of relative percent survival (RPS) compared with control group. Qin et al. [15] also indicated that dietary supplementation with chito-oligosaccharides significantly reduced the inflammatory response in the intestine, which subsequently enhanced the health status and decreased the cumulative mortality of tilapia after challenged with *A. hydrophila.*

IV. CONCLUSIONS

The present study at pond culture provided evidences that dietary COS at 100 mg/kg feed not only considerably improved the growth performance but also reduced the FCR of striped catfish for 9 months of feeding trial. The benefit resulted by COS supplement was manifested by the increase weight gain as well as the improvement of survival rate of striped catfish fed with COS compared to control group. Thus, the COS could be the potential and promising immunostimulant for improving weight gain, survival rate, immune system and controlling disease in fish culture.

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