Fish & Shellfish Immunology 65 (2017) 127–135

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Dietary administration of sodium alginate ameliorated stress and promoted immune resistance of grouper *Epinephelus coioides* under cold stress

Pai-Po Lee [a], Yu-Hung Lin [b], Min-Chieh Chen [b], Winton Cheng [b, *]

[a] Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan, ROC
[b] Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan, ROC

ARTICLE INFO

Article history:
Received 6 February 2017
Received in revised form
7 April 2017
Accepted 13 April 2017
Available online 14 April 2017

Keywords:
Sodium alginate
Grouper
Photobacterium damselae subsp. *piscicida*
Immune parameter
Cold stress
Stress indicator

ABSTRACT

Grouper, *Epinephelus coioides*, fed a diet containing sodium alginate at 0 (control, named C) and 1.0 g kg^{-1} (named S) at a temperature of 28 °C for 12 days, were then further individually transferred to 28 (two groups named C-28 and S-28) or 20 °C (two groups named C-20 and S-20), and immune parameters and stress indexes were measured at the beginning and after 6, 12, 24 and 48 h of exposure. Examination of immune parameters revealed that the alternative complement activity (ACH_{50}), lysozyme activity, phagocytic activity, superoxide dismutase, and respiratory bursts significantly increased in groupers fed the sodium alginate-containing diet for 12 days, and were higher in the S-28 than those of the C-28 and S-20 groups, which were higher than those of the C-20 group from 6 to 48 h except for ACH_{50} at 48 h, respiratory bursts at 48 h, and lysozymes at 6 h. For the assessment of stress indicators, cortisol, glucose, and lactate levels of serum significantly decreased in grouper fed the sodium alginate-containing diet for 12 days, and were higher in the C-20 group than those of the C-28 and S-20 groups, which were higher than those of the S-20 group at 6–48 h. In another experiment, grouper fed the test diet for 12 days at a temperature of 28 °C were challenged with *Photobacterium damselae* subsp. *piscicida* at a dose of 5×10^3 colony-forming units (cfu) (g fish)$^{-1}$, and then individually transferred to 28 or 20 °C. The survival rate of challenged fish of the C-28 group was significantly lower than those of challenged fish of the C-20 and S-28 groups, which were significantly lower than that of challenged fish of the S-20 group. All challenged fish of the S-20 group survived. Survival rates over 144 h were 30.0%, 70.0%, and 56.7% for the C-28, C-20, and S-28 groups, respectively. Our results indicated that dietary sodium alginate administration downregulated stress response indicators, enhanced immune responses, and prevented impacts of physiologic stress responses, immunosuppression, and susceptibility to *P. damselae* subsp. *piscicida* in grouper subjected to cold stress. Grouper cultured at 28 °C were more susceptible to *P. damselae* subsp. *piscicida* infection.

1. Introduction

Groupers, *Epinephelus* spp., are one of the major commercial mariculture fish species in the world. The global production and output of grouper culture have developed rapidly, especially in China and countries of Southeast Asia. The intensive culture of groupers in Taiwan has dramatically developed due to its suitable climate. It is known that rapid degradation of the environment of intensive culture ponds may result in increased incidences of diseases that can lead to culture failure and economic losses. In the past few decades, commercial grouper farming has been severely hit by epidemics associated with viral and bacterial infections. Therefore, the health of fish and enhancement of their anti-stress and immune mechanisms are of primary concern.

Various pathogenic bacteria, including *Vibrio alginolyticus* [1], *V. carchariae* [2], *Pseudomonas* sp. [3], and *Flexibacter* sp. [4], were reported to infect groupers. Arthur and Ogawa [5] indicated that *Streptococcus* spp. is the causative agent of red boil disease in grouper. The halophilic bacterium *Photobacterium damselae* subsp. *piscicida* is the etiological agent of pasteurellosis, which is one of the most threatening diseases of wild and cultured marine fishes

* Corresponding author.
E-mail address: Winton@mail.npust.edu.tw (W. Cheng).

http://dx.doi.org/10.1016/j.fsi.2017.04.007
1050-4648/

and was reported from the USA, Japan, and Mediterranean countries [6]. The disease is also recorded in red grouper, *E. akaera* [6,7].

Environmental fluctuations inducing stress were demonstrated to affect the survival, growth, and physiological and immunological functions of aquatic animals. In teleosts, the primary response to physiological stress involves the release of glucocorticoids and catecholamines that then induce hyperglycemia as a secondary response to deal with the stressor and maintain homeostasis [8]. Cortisol is the major glucocorticoid produced by teleosts [9], and considerable evidence exists that this steroid directly affects the immune system and disease resistance [10]. Temperature fluctuations depend on the weather, and are the most-general stressor of organisms. Young *E. coioides* can grow and survive at temperatures of 15–32 °C, with the optimum at 22–28 °C [11]. Cheng et al. [12] indicated that *E. coioides* reared at 27 °C and then subjected to temperature changes to 19 or 35 °C showed decreased immune responses and increased susceptibility to pathogens.

The use of antibiotics and chemical disinfectants is a traditional strategy of disease control, and has been successfully applied to relieve losses from diseases in hatchery- and pond-cultured fish. However, the popular use of medicines has resulted in drug residuals in food and the spread of antibiotic-resistant pathogens in the aquatic environment, which threaten the safety and hygiene of food, and control of diseases in humans. Some success has been achieved with immunostimulants as a more environmentally friendly approach to disease management [13–15]. Alginic acid is a natural polysaccharide extracted from the cell walls of brown seaweed, which has been used as an immunostimulant in aquaculture to improve non-specific immune responses and disease resistance of aquatic animals like the abalone *Haliotis diversicolor supertexta* [16], shrimp *Litopenaeus vannamei* [17], and grouper *E. coioides* [18] and *E. fuscoguttatus* [19]. Harikrishnan et al. [15] indicated that the overdoses and long-term feeding trial may reduce the efficacy, and therefore, the effective administration period and dosages should be assessed. Dietary sodium alginate administration at 1 and 2 g (kg diet)$^{-1}$ significantly promoted the immunocompetence of grouper after 12 days of feeding trial [18,19], and furthermore, the significantly increased growth performance revealed in grouper fed the diets containing sodium alginate at 1 g (kg diet)$^{-1}$ after 8 weeks of feeding trial [18]. We assumed that alginic acid as an immunostimulant could relieve the stress responses of grouper, and further lead to decreased immunosuppression and susceptibility to pathogen infections, which benefit the growth performance of grouper.

Non-specific immune systems are very important in the defense mechanisms of fish against pathogens and microorganisms. The goals of this study were to determine the parameters of physiological stress and innate immune responses in the grouper, *E. coioides,* and its resistance against *Pho. damselae* subsp. *piscicida* when fish were fed a sodium alginate-containing diet for 12 days and then subjected to hypothermal stress.

2. Materials and methods

2.1. Diet preparation

Two diets containing different levels of sodium alginate were prepared as described in Table 1. The basal diet contained 0.1% cellulose, which served as the control diet. Proximate analysis of the basal diet revealed 51.3% crude protein, 8.4% crude lipid, 12.5% ash, and 9.7% moisture. Sodium alginate (Kimitsu Algina I-1, Kimitsu Chemical Industries, Chiba, Japan) was added to the test diets at a level of 1.0 g (kg diet)$^{-1}$ with a corresponding decrease in the amount of cellulose. The ingredients were ground up in a Hammer mill to pass through a 60-mesh screen. Experimental diets were prepared by adding water until a stiff dough resulted. Each diet was then passed through a mincer with a die, and the resulting spaghetti-like strings were dried in a drying cabinet using an air blower at 40 °C until the moisture levels were at around 10%. After drying, the finished pellets were stored in plastic bins at 4 °C until use.

Table 1
Composition of the basal diet administrated with sodium alginate.

Ingredients	Sodium alginate in diets (g kg^{-1})	
	0	1
Fish meal	500	500
Soybean meal	120	120
Squid	80	80
α-starch	200	200
Gluten	50	50
Pre-Mix	50	50
sodium alginate	0	1
cellulose	1	0

2.2. Culture of pathogens

The bacterial pathogen used in the present study, *Pho. damselae* subsp. *piscicida,* was provided by Dr. J. P. Shu, chairman of the Animal Health Inspection and Quarantine Institute, Pingtung County, Taiwan, which had been isolated from a diseased giant grouper with symptoms of a darkened body color, hemorrhagic inflamed anus, and white granulomatosis in the liver. Stocks cultures were plated on blood agar for 16 h at 28 °C, and then transferred to 10 ml of tryptic soy broth (TSB supplemented with 2% NaCl, Difco) for 16 h at 28 °C as the culture for the test groups. The broth culture was centrifuged at 8000×*g* for 10 min at 4 °C. The supernatant was removed, and the bacterial pellet was suspended in a saline solution at a concentration of 5×10^6 colony-forming units (cfu) ml^{-1} for the susceptibility test.

2.3. Experimental design

Juvenile grouper, *E. coioides*, purchased from a private farm in Pingtung, Taiwan, were shipped to our laboratory. The fish were acclimated indoors in a 2-metric ton tank with recirculating aerated seawater (25‰) at 28 ± 1 °C, and fed the control diet (without sodium alginate) for 2 weeks before the experiment. Seawater was adjusted with heater or cooler to the desired temperatures of 20, and 28 °C.

There were five treatments for the susceptibility test, including one unchallenged control group, and two diets of 0 and 1.0 g sodium alginate (kg diet)$^{-1}$ combined with two temperatures of 20 (named the C-20 and S-20 groups, respectively) and 28 °C (named the C-28 and S-28 groups, respectively). Each treatment consisted of three tanks as triplicates, and each replicate was comprised of ten fish kept in a 0.5-metric ton FRP tank containing 0.4 metric ton of aerated seawater (25‰). To examine physiologic parameters and non-specific immune parameters, there were four treatments including two diets of 0 and 1.0 g sodium alginate (kg diet)$^{-1}$ combined with two temperatures of 20 and 28 °C. In all tests, fish were fed the test diets twice daily. Average weights of the fish were 19.5 ± 0.5 g for the challenge trails and 27.8 ± 2.3 g for the physiological and immunological parameter analyses. No significant differences in weight were observed among treatments. During the experiments, 50% of the seawater was exchanged daily to maintain the water quality, and the water temperature was maintained at 28.0 ± 0.5 °C, the pH at 7.6–8.6, and the salinity at 25‰.