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Efficacy of probiotics, prebiotics, and immunostimulant on growth performance and immunological parameters of *Procambarus clarkii* juveniles

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KEYWORDS

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Abstract *Procambarus clarkii* juveniles were used as an aquatic model to investigate growth performance and immune parameters after 6 weeks of feeding with supplementation of ten experimental diets containing 1%, 2%, 3% Biogen® (as probiotics), *Allium sativum* (garlic) and *Cynodon dactylon* (as immunostimulant) and one concentration (3 g/L) of sodium alginate (as prebiotics), compared with feeding on control basal diets only. All supplementation diets increased survival rates and wet weight, while, 1% and 2% *C. dactylon* showed a significant ($P \leqslant 0.05$) increase in weight gain percentage (WG%), and specific growth rate (SGR). Feeding with diet containing 2% Biogen®, 2% and 3% garlic and sodium alginate showed a significant ($P \leqslant 0.05$) increase in SGR rate after 6 weeks. Synergetic effect of 1% and 3% Biogen® and sodium alginate in total hemocytes count (THC) which increased significantly ($P \leqslant 0.05$) after 6 weeks of feeding. Prophenoloxidase activity increased with all supplemented diets. While SOD increased significantly with 3% *C. dactylon*, 1% and 3% garlic and 2% Biogen. The results indicated that Biogen®, garlic, *C. dactylon* and sodium alginate inclusion with a basal diet had the potential to improve the growth and immune response of *P. clarkii* juveniles, hence this enables us to use the three supplementation diets in fish and prawn farms to improve their growth and immune parameters.

Introduction

Aquaculture has a long history that can be traced back for more than 2000 years (Boyd and Tucker, 1998). Under intensive production systems, fish and crustaceans are exposed to various stressful conditions leading to growth reduction, immunosuppression and susceptibility to infectious diseases resulting in a major economic loss for farmers. The potential for reducing stress and enhancing immunity and disease resistance by nutritional factors/feed additives such as probiotics/prebiotics, immunostimulants and bacterins has been demonstrated in warm-blooded animals, fishes and crustacea.

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During the past 50 years, numerous trials were conducted with microorganisms known as probiotics in efforts to improve culturability of food species, and to improve human health and welfare. Several probiotic species were used, including *Lactobacillus* spp, *Saccharomyces* sp., *Bacillus* spp, and mixed cultures (Muralidhara et al., 1977; Ozawa et al., 1981; Lessard and Brisson, 1987; Surawicz et al., 1989). The use of immunostimulants is being introduced into fish farming routine procedures as a prophylactic measure. These substances haven't any negative side effects that live vaccines and antibiotics may have on consumers and on the environment, and are generally classified as biological response modifiers (Anderson, 1992; Secombes, 1994).

Probiotics are dietary supplements containing potentially beneficial live bacteria, yeast or algae, which when consumed in adequate amounts confer a health benefit for the host (FAO/WHO, 2001). Immunostimulants such as garlic (*Allium sativum*) and bremuda grass (*Cynodon dactylon*) increase the immune responses in several shrimp species in aquaculture by promoting phagocytosis, bactericidal activity, proPO activity, and respiratory bursts, and enhancing resistance against pathogens (Chang et al., 2000). Garlic increases fish welfare, and is able to support the control of pathogens, especially bacteria and fungi (Corzo-Martinez et al., 2007). *C. dactylon* inclusion with a basal diet had the potential to improve the growth and immune response of prawns *Macrobrachium rosenbergii* (El-Desouky et al., 2012). Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of a limited number of bacteria, usually bifidobacteria and lactobacilli, and thus improve host health (Gibson and Roberfroid, 1995). Growth performance and immunological parameters could be increased on using of probiotics, prebiotics and immunostimulants as daily supplementary diets (Cheng et al., 2005; Ziaei-Nejad et al., 2006; Diab et al., 2008; Ndong and Fall, 2011; El-Desouky et al., 2012; Poongodi et al., 2012).

Procambarus clarkii (the red swamp crayfish), is widely used in aquaculture and represents as an important food source because it is highly adaptable inhabiting a wide range of aquatic environments (Girard, 1852). *P. clarkii* can withstand extreme environmental conditions (Gherardi, 2007) and is able to colonize varied ecosystems as it affects both lower and higher trophic levels, by grazing on macrophytes and preying on macroinvertebrates, and has a role as an important food source for several vertebrate species. In addition to being a vector of the crayfish plague, due to its short life-history, rapid growth, burrowing activities and high population density, it can adversely impact the aquatic environment (Gherardi, 2006; Gherardi and Acquistapace, 2007).

Supplementary diet administration of Biogen® as probiotics, Garlic and *C. dactylon* as immunostimulant and sodium alginate as prebiotic, was evaluated on growth performance every week for 6 weeks and immunological parameters as Total hemocytes count (THC). Prophenoloxidase (proPO) and Superoxide dismutase activity (SOD) of *P. clarkii* juveniles after 6 weeks feeding trial.

Materials and methods

Experimental animals

330 Juveniles of *P. clarkii*, were obtained by size from brackish water in EL-Fayoum governorates, Egypt. Crayfish were housed in glass containers (60 L capacity), for 2 weeks before the beginning of the experiment. After acclimatization, individuals were distributed randomly into 33 glass aquaria (30 L capacity) at an initial capacity of ten juveniles per aquarium (tank). The water was changed every two days, including faeces and remaining food, then, each aquarium was refilled to a fixed volume using stored (dechlorinated) and well-aerated freshwater. The air-conditioner was installed in the environment-controlled laboratory maintained at 25 °C.

Experimental diets

Biogen® is a commercial probiotic, and consists of *Bacillus licheniformis* and *Bacillus subtilis* (Diab et al., 2008), constants were as follows: *Allicin* (not less than 0.247 Mmol g^{-1}), *B. subtilis* Natto (not less than 6×10^7 g^{-1}) and *High Unit Hydrolytic Enzyme* (not less than 3690 U g^{-1}). Powdery forms of Garlic, *C. dactylon*, and sodium alginate were used. Prebiotics as sodium alginate (Wang et al., 2006) is a polyuronic saccharide that is isolated from the cell walls of brown seaweed (Brownlee et al., 2005). Artificial basal diet was fish grower consists from wheat flour, cod liver oil (Universal Medicare Pvt. Ltd.) and vitamin premix (Vetsfarma Ltd.), which contains 20% carbohydrates; 41% proteins; 15% lipids and 9% ash.

Table 1 The wet weight change (g) of *P. clarkii* juveniles within 6 weeks of feeding with different food supplements.

Weight	W0	W1	W2	W3	W4	W5	W6
Control	6.55 ± 0.5614	6.824 ± 0.54	6.83 ± 0.59	6.9 ± 0.3	7.07 ± 0.27	7.05 ± 0.54*	7.5 ± 0.1
Biogen 1%	7.407 ± 0.285	7.36 ± 0.35	7.4 ± 0.36	7.97 ± 0.2*	8.10 ± 0.27*	8.8 ± 0.49*	8.85 ± 0.6*
Biogen 2%	5.793 ± 0.66	5.8 ± 0.617	5.8 ± 0.61	6.64 ± 0.9*	6.95 ± 1.07*	7.16 ± 1.11*	7.24 ± 1.2*
Biogen 3%	8.083 ± 0.565	8.23 ± 0.567*	8.16 ± 0.69	8.5 ± 0.7	8.22 ± 0.51	8.48 ± 0.38	8.3 ± 0.1
Garlic 1%	7.54 ± 0.89	7.675 ± 0.93*	8.366 ± 0.6	8.41 ± 0.5	8.71 ± 0.33	8.49 ± 0.8	8.51 ± 0.3
Garlic 2%	5.65 ± 0.687	5.85 ± 0.557	6.203 ± 0.739*	6.62 ± 1.01*	6.5 ± 0.67*	6.85 ± 0.8*	6.85 ± 0.9*
Garlic 3%	3.726 ± 0.89	4.57 ± 0.59*	4.911 ± 0.15*	4.45 ± 0.9*	4.53 ± 0.91*	4.44 ± 0.9*	4.65 ± 1.03*
Cynodon dactylon 1%	4.77 ± 0.26	4.97 ± 0.44	5.81 ± 1.07*	6.67 ± 0.87*	6.6 ± 0.14*	6.2 ± 0.5*	6.58 ± 0.4*
Cynodon dactylon 2%	3.917 ± 0.26	3.89 ± 0.5	5.011 ± 0.804	4.73 ± 0.9	5.44 ± 0.8*	5.24 ± 0.2*	5.22 ± 0.4*
Cynodon dactylon 3%	5.072 ± 0.14	5.48 ± 0.36	5.23 ± 0.2	5.68 ± 1.07*	5.59 ± 0.3*	5.72 ± 0.29	5.77 ± 1.12
Sodium alginate	3.726 ± 0.89	4.57 ± 0.59	4.911 ± 0.15	4.45 ± 0.9	4.53 ± 0.91	4.44 ± 0.9	4.65 ± 1.03*

* $P \leqslant 0.05$.