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Research Article

Symbiotic effect of *Bacillus clausii* and Galacto-oligosaccharide on growth and survival rates in red cherry shrimp (*Neocaridina davidi*)

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ABSTRACT

The effect of probiotics (*Bacillus clausii*, commercial probiotics: Enterogermina) and prebiotics (Galactooligosaccharides, GOS) on the growth of red cherry shrimp (*Neocaridina davidi*) has been investigated for 30 days. The trial was conducted with 270 red cherry shrimps (initial weight: 0.24±0.03 g) in 27 plastic tanks (volume 0.01 m^3 and area of 0.05 m^2) representing 9 groups. Except for the control group, diets were supplemented with either GOS or synbiotics per kg of feed as G1 (1g GOS), G2 (2g GOS), G3 (3g GOS), G4 (4g GOS), G1P (1g GOS+ 1 ml probiotic), G2P (2 g GOS+ 1 ml probiotic), G3P (3 g GOS+ 1 ml probiotic) and G4P (4 g GOS+ 1 ml probiotic). This study showed significantly ($P<0.05$) higher growth rate indices of male and female shrimps fed with feed additives groups compared to the control group. The highest final body weight (FBW), weight gain (WG), and specific growth rate (SGR) in male shrimps were recorded from the 2 g GOS+ 1 ml probiotic and 3 g GOS+ 1 ml probiotic groups. Similarly, the highest FBW, WG and SGR in the female shrimps were found in the G1P and G2P synbiotic groups. Conversely, the lowest FBW, WG and SGR of both the female and male groups were noted in the control groups. Regarding feed conversion ratio (FCR) and survival rates of both female and male shrimps, G2P and G3P groups showed significant improvements. Our study's findings stated that 2 g GOS and 1 ml of *Bacillus clausii* (commercial probiotic: Enterogermina) dietary synbiotics can be used to improve red cherry shrimp growth rates.

Introduction

The production of crustaceans in aquaculture has increased from 7 million tons, which is about 36 billion US dollars in 2014 (FAO, 2016), to 11.3 million tons, which is estimated at 81.5 billion US dollars in 2020 (FAO, 2022). This dramatic increase, which is expected to continue growing, is not just for human consumption as crustaceans are grown for other reasons, one of which is aquarium sector (Sganga et al., 2019). The cultivation of ornamental crustaceans is an established industry that provides huge economic incentives to both small and large-scale farmers and traders. This is because crustaceans have a variety of beautiful and bright colors that have become attractive for many aquarium hobbyists (Amaya and Nickell, 2015). Red cherry shrimp (*N. davidi*) is one of the commonly used ornamental crustaceans. The species is a native of far east Asian countries such as China, Taiwan, Korea, Vietnam, and Japan, and is a member of the family Atyidae, a cosmopolitan shrimp family readily available in tropical, subtropical, and temperate regions (Yixiong, 1996). *N. davidi* can be easily kept in captivity, survive on less or minimal feeding, and can tolerate varying temperatures and higher stock densities (Pantaleão et al., 2015). Red cherry shrimp have also a short life cycle and a very good reproduction pattern producing several spawns with the same brood quality (Sganga et al., 2018).

Currently, research focuses on improving both the quantity and quality of aquaculture feeds using functional feed additives and immunostimulants. Especially the feeds formulated for crustaceans, which, unlike fish, are known to be unable to develop acquired immunity and therefore their innate immunity should constantly be stimulated with immunostimulants (Kaya et al., 2020). In addition, feeds supplemented with such feed additives improve growth, reproduction, egg quality, and shrimp welfare (Shehata et al., 2022). Some of the functional feed additives used in aquaculture are probiotics and

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prebiotics, both of which are applied in this study. Probiotics are beneficial microorganisms composed mainly of bacteria and yeast that can modulate the digestive systems of the farmed aquatic animal; prebiotics are indigestible fibres that support the growth and proliferation of probiotics. (Quigley, 2019). Among the frequently used prebiotics in aquaculture are mannan oligosaccharides (MOS), fructooligosaccharides (FOS, and galactooligosaccharides (GOS). In particular, GOS obtained from the enzymatic conversion of lactose has received significant research focus due to its various benefits (Ringø et al., 2010). Bacillus clausii has been evaluated one of the potential probiotics because of its antagonistic activity against fish pathogens (Yang et al., 2012) and it has been reported that this probiotic has a synergistic effect with prebiotics (Ye et al., 2011).

In aquaculture, both probiotics and prebiotics are used to improve aquatic animal growth, feed utilization, tissue histomorphology, disease resistance, immune response, and animal welfare. In crustaceans, prebiotics have been reported to modulate digestive enzymes, improve digestibility, growth performance, feed efficiency, enhance survival, and resistance to pathogens, and improve intestinal bacterial load and water quality of different shrimp species; whiteleg shrimp (*Litopenaeus vannamei*) (Luis-Villasenor et al., 2013; Nimrat et al., 2021; Mirbakhsh et al., 2022), giant tiger shrimp (*Penaeus monodon*) (Karthik et al., 2014; Kolanchinathan et al., 2022), giant freshwater prawn (*Macrobrachium rosenbergii*) (Mohamad et al., 2020), and fleshy prawn (*Fenneropenaeus chinensis*) (Chai et al., 2016). Similarly, many authors have also shown the beneficial effect of GOS on different shrimp (Huynh et al., 2018; Fu et al., 2019) and crayfish species (Nedaei et al., 2019). It has also been proven that the mixture of prebiotics and probiotics, called synbiotics, has a synergistic effect and can improve the biochemical and growth parameters of aquatic animals. Many studies have emphasized that the administration of prebiotics or probiotics alone is generally less effective than their combined forms (Ai et al., 2011; Lee et al., 2018). This is especially true where probiotic microorganisms face difficulties in surviving in the digestive tract, so careful selection of combined prebiotics and probiotics is considered necessary (Pandey et al., 2015). Many research articles on the effect of synbiotics on aquatic animals in general and crustaceans, in particular, have been conducted in recent years (Huynh et al., 2018; Butt et al., 2021). Nevertheless, there is a lack of information regarding the possible effects of prebiotics, probiotics, and their combinations on ornamental shrimp, particularly red cherry shrimp. In this trial, the effects of synbiotics: prebiotics (GOS) and probiotics (*Bacillus clausii*, commercial probiotics: Enterogermina, Sanofi, Istanbul, Turkey) on the growth rate indices of red cherry shrimp (*Neocaridina davidi*) were investigated.

Materials and Methods

Experimental Design

Red cherry shrimp obtained from a local producer (Eker Ornamental Prawns Production Farm, Antalya, Türkiye) was brought to Ankara in a plastic transport tank (10 L) with an aeration system. The trial was conducted in the Aquaculture Research Unit (Department of Fisheries and Aquaculture Engineering, Ankara University, Turkey). In this study, red cherry shrimps with an initial weight of 0.24±0.03 g in total 270 were used. The shrimp were allotted to the plastic tanks with a volume of 10 L (0.01 m^3) and an area of 0.05 m^2, representing 9 groups, each of which contained 3 replicates and ten shrimps were placed in each tank. Except for the control group, diets for all shrimps were supplemented with either GOS or synbiotics. Plastic shelters and algae were used for shrimp hiding spots. To maintain good water quality siphoning of unconsumed feed and shrimp excrement was ensured. The temperature was kept at the optimum level; a warm air blower (2000W, Kumtel, Istanbul, TR) was used in case of a temperature drop, while the photoperiod was ensured to be as 12 hours of light and 12 hours of darkness. During this thirty-day experiment, dissolved oxygen, temperature, and pH were recorded on daily basis using YSI Pro20 for oxygen and temperature and YSI EcoSense pH100A for pH measurement. Further, total ammonia was also measured each week using an Iris Visible Spectrophotometer - HI801-01 and the data obtained were analyzed with SPSS. Water quality parameters were 6.4±0.3 mg/L, 26±1°C, 6.6±0.2, and 0.3±0.1 mg/L for dissolved oxygen, temperature, pH and ammonia, respectively.

Experimental diets

The basal diets (2-3 mm, Novoprawn Perlgarnelenfutter, JBL GmbH & Co. Neuhofen, Germany) contained 38.4% crude protein, 10.4% lipid, 6.9% ash, 10% moisture, and 1.9% cellulose. In the experiment, probiotics as Enterogermina (1 ml, 2 x 10^9 CFU Bacillus clausii, www.enterogermina.com.tr) and/or the Galactooligosaccharide as prebiotics (GOS, Vivinal GOS, Friesland Foods Domo, Zwolle, The Netherlands) were added in the basal diets. The feeds of the 9 groups were supplemented with the following feed additives (per kg): control (0 g), G1 (1 g GOS), G2 (2 g GOS), G3 (3g GOS), G4 (4g GOS), G1P (1 g GOS+ 1 ml probiotic), G2P (2 g GOS+ 1 ml probiotic), G3P (3 g GOS+ 1 ml probiotic) and G4P (4 g GOS+ 1 ml probiotic). All groups were fed with these feeds twice a day until satiation (ad libitum), and faeces with remained feed were removed by siphoned from the tanks after feeding.

Growth Performance

During the experiment, the total weight of all shrimps after the shrimps are dried with paper towels was measured using a precision balance (Radwag brand PS 360.R2 model) once in two weeks intervals. Shrimp's sexual dimorphism cannot be detectable in the juvenile stage. At the end of the experiment, all the individuals reached maturity and the female and male counting and weighting were performed. 24 hours before each measurement feeding was stopped to ensure obtaining dry weights. At the experiment, a digital meter (balance accurate to 0.001 g) was used for the growth parameters. The growth indexes were determined using the below formulas:

WG (Weight gain, g): Final weight of shrimps (g)-Initial weight (g)

FCR (Feed conversion ratio): Feed given to shrimps (g)/body weight gain (g)

SR (Survival rate, %): (The number of shrimps at end of trial/ initial number of shrimps) x 100

SGR (Specific growth rate, %/day): (ln final shrimp weight)-(ln initial shrimp weight)/trial day x 100

Statistical Analysis

Data collected for growth and water quality indices after the end of the experiment were analyzed using the package program (SPSS 17.0, Chicago, IL, USA). Tukey, a post hoc ANOVA test, was chosen to examine the difference among the groups. While the P value was determined as 0.05, the findings were given as the mean ± standard deviation.

Results

At the end of the experiment, female ratio was 52.15±0.41, 51.80±0.36, 52.02±0.42, 53.01±0.45, 51.65±0.48, 52.70±0.34, 54.04±0.49, 51.80±0.21 and 52.60±0.40 for the G1, G2, G3, G4, G1P, G2P, G3P and G4P groups, respectively. The findings of growth rate indices of red cherry shrimp fed with diets supplemented with various doses of Galactooligosaccharides and Enterogermina probiotics are summarized in Table 1. In general, the growth parameters were found to be significantly higher ($P<0.05$) in shrimp fed with prebiotic and synbiotic supplemented feeds compared to shrimps in control, regardless of the male or female groups. The highest FBW in male groups was recorded from the groups fed with synbiotic added feeds (G2P: 0.454±0.048 g and G3P: 0.437±0.050 g), while the lowest FBW was noted from shrimp in the control (0.404±0.047 g) and G1 (0.411±0.039 g) groups. Similarly, the highest FBW in female groups was found in synbiotic groups (G1P: 0.690±0.055 g and G2P: 0.716±0.039 g) while the lowest was recorded from shrimp in the control group shrimps. In terms of WG and SGR, the highest values in both male and female groups were found in the G2P groups, while the lowest values were recorded in the control groups ($P<0.05$). For FCR of male groups, synbiotic supplemented shrimps (G2P: 1.820±0.072 and G3P: 1.933±0.153) depicted the lowest values ($P<0.05$) compared to other groups, while in female groups G2P (1.797±0.148) showed the best FCR value. Although there were no statistical differences ($P<0.05$) were noted among the female groups in terms of survival rate, the lowest survival rates were recorded in the control group. On the contrary, significant differences ($P<0.05$) were found in the male control group (73.33±23.09%) with the lowest survival rate when compared to the G2, G3, G2P, and G3P groups, which showed 100% survival rates.

Discussion

Synbiotics, a combination of probiotics and prebiotics that are often in a synergistic relationship, have recently become a magnet for many research studies focusing on the use of functional feed additives in aquaculture (Butt et al., 2021). Prebiotics, such as Galactooligosaccharides, has been proven to promote the growth and development of commensal bacteria that are distributed through the digestive enzymes of aquatic animals (Grisdale-Helland et al., 2008; Nedaei et al., 2019). The selection of probiotics is usually determined based on their ability to improve the biochemical and zootechnical parameters of aquatic animals, while the selection of prebiotics is carried out in accordance with their ability to stimulate the growth and proliferation of probiotic microorganisms (Kolida and Gibson 2011). When probiotics are selected for suitable prebiotics, many studies have reported that a combination (synbiotics) performs better than either prebiotics or probiotics alone (Merrifield et

Table 1. Dietary synbiotics applications on growth rate indices of red cherry shrimp

		Control	G1	G2	G3	G4	G1P	G2P	G3P	G4P
M	IW	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}
	FBW	0.404±0.047^{a}	0.411±0.039^{a}	0.418±0.029^{ab}	0.413±0.030^{ab}	0.408±0.034^{a}	0.428±0.037^{bc}	0.454±0.048^{c}	0.437±0.050^{c}	0.419±0.053^{ab}
	WG	0.167±0.058^{a}	0.193±0.056^{a}	0.201±0.039^{ab}	0.196±0.047^{ab}	0.171±0.048^{a}	0.243±0.044^{bc}	0.269±0.063^{c}	0.251±0.055^{bc}	0.197±0.065^{ab}
	SGR	0.885±0.293^{a}	0.996±0.287^{ab}	1.027±0.213^{ab}	1.009±0.253^{ab}	0.907±0.257^{a}	1.180±0.216^{bc}	1.263±0.283^{c}	1.204±0.245^{bc}	1.015±0.312^{ab}
	FCR	2.617±0.215^{c}	2.293±0.276^{bc}	2.247±0.514^{bc}	2.400±0.087^{bc}	2.060±0.122^{ab}	2.000±0.009^{ab}	1.820±0.072^{a}	1.933±0.153^{a}	2.050±0.050^{ab}
	SR	73.33±23.09^{a}	86.67±11.55^{ab}	100.0±0.00^{b}	100.00±0.00^{b}	93.33±11.55^{ab}	93.33±11.55^{ab}	86.67±23.09^{ab}	100.00±0.00^{b}	100.00±0.00^{b}
F	IW	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}	0.242±0.027^{a}
	FBW	0.613±0.046^{a}	0.619±0.041^{ab}	0.624±0.033^{ab}	0.622±0.053^{ab}	0.621±0.054^{ab}	0.690±0.055^{c}	0.716±0.039^{c}	0.628±0.065^{ab}	0.675±0.047^{bc}
	WG	0.371±0.064^{a}	0.377±0.053^{a}	0.382±0.050^{ab}	0.380±0.056^{ab}	0.379±0.055^{a}	0.448±0.069^{bc}	0.474±0.056^{c}	0.386±0.069^{ab}	0.433±0.065^{ab}
	SGR	1.555±0.275^{a}	1.572±0.230^{a}	1.586±0.230^{a}	1.577±0.214^{a}	1.573±0.204^{a}	1.751±0.260^{ab}	1.815±0.240^{b}	1.591±0.241^{a}	1.715±0.260^{ab}
	FCR	2.847±0.243^{d}	2.108±0.251^{ab}	1.953±0.525^{ab}	2.470±0.108^{bc}	2.393±0.361^{bc}	1.995±0.009^{ab}	1.797±0.148^{a}	2.100±0.200^{ab}	2.017±0.104^{ab}
	SR	80.00±20.00^{a}	93.33±11.55^{a}	93.33±11.55^{a}	93.33±11.55^{a}	93.33±11.55^{a}	100.0±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	93.33±11.55^{a}

IW: Initial weight (g); FBW: Final body weight (g); WG: Weight gain (g); SGR: Specific growth rate (%/day); FCR: Feed conversion ratio; SR: survival rate (%). C (0 g), G1 (1 g GOS), G2 (2 g GOS), G3 (3g GOS), G4 (4g GOS), G1P (1 g GOS+ 1 ml probiotic), G2P (2 g GOS+ 1 ml probiotic), G3P (3 g GOS+ 1 ml probiotic) and G4P (4 g GOS+ 1 ml probiotic). Results are depicted as mean ± standard deviation. Different superscript in the same row shows a considerable difference ($P<0.05$).

al., 2010). In fact, many researchers argue that in the shrimp industry synbiotics are more effective than the sole application of probiotics or prebiotics (Oktaviana and Yuhana 2014; Zhang et al., 2011; Hamsah et al., 2019).

Although many studies on the effects of prebiotics and probiotics on aquatic animals have been performed in recent years, to the best of our knowledge this is the first study to determine the impacts of synbiotics (probiotics (Enterogermina probiotic- *B. clausii*) and prebiotics (GOS)) on the growth and survival of red cherry shrimp (*N. davidi*). Prebiotics such as GOS, MOS, and FOS have been combined with different Bacillus species and supplemented with various fish and crustacean species; Positive effects on growth, immunity, tissue histomorphology and gut microbiota have been reported from these studies (Daniels et al., 2010; Ai et al., 2011; Kaya et al., 2021). A recent study conducted by Modanloo et al., (2017) found that synbiotics (*B. clausii* and FOS, GOS, and MOS) improved the immune response of Japanese flounder (*Paralichthys olivaceus*).

Our results showed that, regardless of male or female red cherry shrimp, the groups supplemented with feed additives (GOS and/or synbiotics) showed improved growth rates compared to the control group. Similar results have been reported from white leg shrimp (*L. vannamei*) fed GOS-supplemented diets compared to their control counterparts in different studies (Huynh et al., 2018; 2019). We found that the highest FBW in male and female groups were recorded from the shrimp fed with synbiotic added feeds (G2P: 0.454±0.048 g and G3P: 0.437±0.050 g) and (G1P: 0.690±0.055 g and G2P: 0.716±0.039 g) respectively. This means that synbiotics have demonstrated a synergistic effect, which is consistent with the findings of Hamsah et al., (2019), who found better results in immunity and growth of white leg shrimp when fed to diets added with synbiotics (MOS and *Pfiesteria piscicida*) instead of probiotics or prebiotics alone. Furthermore, the beneficial effect of synbiotics on the growth rate parameters of different shrimp species was reported; Pacific white shrimp (*L. vannamei*) (Yu et al., 2009; Munaenı et al., 2014; Huynh et al., 2018); Kuruma prawn (*Penaeus japonicus*) (Zhang et al., 2011) and Tiger shrimp (*P. monodon*) (Bir et al., 2020). The improved growth performance of shrimp fed diets including synbiotics can be linked to increased feed utilization. According to our results, the lowest feed conversion rate (FCR) was obtained in the group the male red cherry shrimp (1.820±0.072) and in the female (1.797±0.148) in the synbiotic fed shrimp groups. Although there were no significant differences between the groups of female and male red cherry shrimp in terms of survival rates, higher values were recorded in the shrimp that were fed diets added with synbiotics. In contrast, Hamsah et al., (2019) reported that Pacific white shrimp fed with synbiotic-supplemented feeds showed significantly higher survival rates compared to their control groups. Finally, in agreement with the results of Yu et al., (2009) who noted that synbiotics improved WG and SGR of Pacific white leg shrimp, our findings indicated considerably higher specific growth rates in the red cherry shrimp which fed to diets added with synbiotics and GOS supplementation.

Conclusion

In this study, results indicated significantly higher growth rate parameters in all the red cherry shrimps which fed supplemented diets with prebiotics (GOS) and synbiotics (GOS and *B. clausii*) than the control group shrimps. Furthermore, both females and males of red cherry shrimp groups which fed with synbiotics(G2P) showed the best performance in all growth parameters, so it is better to recommend adding synbiotic (2 grams of GOS and 1 ml of probiotic Enterogermina) into feed for red cherry shrimp. Finally, more studies needed in terms of histomorphology, body composition, and pigmentation on the effect of synbiotics on red cherry shrimp (*N. davidi*).

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COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

Authors contributed equally to this paper.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

The authors followed all applicable international, national, and/or institutional guidelines for the care and use of animals.

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