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**ARAŞTIRMA MAKALESİ** **RESEARCH PAPER**

# The Effects of Dietary GroBiotic®-A on Growth Performance and Survival Rate of Green Tiger Shrimp *Penaeus semisulcatus* (de Haan, 1844) Juveniles

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**Abstract:** In this study, the effects of GroBiotic®-A on growth performance, survival rate, and feed conversion ratio of green tiger shrimp were investigated. The commercial GroBiotic®-A was added to the commercial sea bass feed diet at four different levels (0, 0.5, 1, and 2%). *Penaeus semisulcatus* juveniles with an average weight of 1.65±0.08 g was stocked randomly in 0.785 $m^2$ cylindrical tanks and grown for 45 days. The experiment consisted of four treatment groups, each with three replications. Although the survival rate and weight gain were found highest in the group with 2% GroBiotic®-A supplementation, no difference was found (P>0.05) among the treatment groups. The feed conversion rate was best observed in the control and 2% GroBiotic®-A supplemented group, but it was found to be statistically insignificant (P>0.05). According to the results of the current study, when the growth, survival rate and feed conversion ratio are taken into account, it is thought that adding 2% GroBiotic®-A to shrimp feed will be beneficial for juvenile culture.

**Keywords:** Green tiger shrimp, *Penaeus semiculcatus*, prebiotic, feed additive, shrimp, growth performance, culture.

# GroBiotic®-A'nın *Penaeus semisulcatus* (de Haan, 1844) Yavrularının Büyüme Performansı ve Hayatta Kalma Oranı Üzerindeki Etkileri

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**Öz:** Bu çalışmada Grobiyotik-A'nın yeşil kaplan karidesinin (*Penaeus semisulcatus*) büyüme, yaşama oranı ve yem değerlendirme oranı üzerine etkileri araştırılmıştır. Deneme yemleri, ticari levrek yemine 4 farklı düzeyde (% 0, 0,5, 1 ve 2) Grobiyotik-A ilave edilerek hazırlanmıştır. Ortalama ağırlığı 1,65±0,08g olan *Penaeus semisulcatus* yavruları 0,785 $m^2$'lik silindirik tanklarda 10 adet olarak stoklanmış ve 45 gün boyunca yetiştirilmiştir. Deneme, her biri üç tekerrürlü dört muamele grubundan oluşturulmuştur. Deme sonunda yaşama oranı ve ağırlık kazancı en yüksek %2 Grobiyotik-A ilaveli grupta gözlenmiş olmakla birlikte, önemli fark bulunmamıştır (P>0,05). Yem değerlendirme oranı en iyi kontrol ve %2 Grobiyotik-A ilaveli gruplarda gözlenmiş fakat istatistiksel olarak önemsiz bulunmuştur (P>0,05). Mevcut çalışmanın sonuçlarına göre büyüme, yaşama oranı ve yem değerlendirme oranı dikkate alındığında karides yemlerine %2 Grobiyotik-A ilavesinin faydalı olacağı düşünülmektedir.

**Anahtar kelimeler:** Karides, *Penaeus semiculcatus*, prebiyotik, yem katkısı, karides, büyüme performansı, yetiştiricilik.

## INTRODUCTION

In order to meet the food needs of the world population, which is predicted to reach approximately 10 billion by 2050, the search for alternative food sources or the efforts to make optimum use of existing sources have gained momentum (FAO, 2018). Fishery products obtained through fishing or aquaculture are one of the lowcost sources of protein and essential nutrients (Hoseinifar et al. 2020a). However for providing maximum return in aquaculture enterprises, maximum utilization of the unit area has been made. Aquaculture in an intense environment leads to poor water quality and malnutrition, which causes stress in fish and increases their susceptibility to disease (Dinardo et al., 2020; Syahidah et al., 2015; Wang et al., 2016). Antibiotics have been used successfully for a long time as the most common method of struggle against the disease problem in aquaculture. However, the emergence of resistant bacterial strains over time and the awareness of their potential harmful effects on the environment, fish and human health triggered the search for alternative products to antibiotics (Caipang et al., 2019; Cunha et al., 2018; Dawood et al., 2021; Romero et al., 2012).

The use of feed additives in aquatic diets, which has increased in recent years, is effective in both increasing the efficiency of feed and reducing stress by strengthening the immune system of the fish, thus controlling diseases (Hoseinifar et al., 2020b; Nawaz et al., 2018; Alnaiem et al., 2021). With the use of these substances, very promising results have been obtained in increasing the efficiency of aquaculture in recent years (Ashouri et al., 2020; Hoseinifar et al., 2021; Miandare et al., 2016; Safari and Sarkheil 2018; Yousefi et al. 2020). Diets prepared with functional feed additives play an important role not only in providing the necessary nutrients for the growth and development of the animals being farmed, but also in protecting the health of the animals raised by increasing their resistance to stress and disease-causing factors (Li & Gatlin, 2004; Rossi et al., 2015; Wang et al., 2016). Prebiotics are considered ideal feed additives because they are completely natural, relatively inexpensive, and can be used for long periods of time to exert their beneficial effects (Raggi et al., 2012). In this respect, prebiotics have offered an important potential in aquaculture.

Prebiotics are considered ideal feed additives because they are completely natural, relatively inexpensive, and can be used for long periods of time to exert their beneficial effects (Raggi et al., 2012). In this respect, prebiotics have offered an important potential in aquaculture. The most commonly used prebiotics in Aquculture are MOS (milk oligosaccharides), FOS (fructo-oligosaccharides), scFOS (short-chain fructo-oligosaccharide), GOS (galacto-oligosaccharides), IMOS (isomalto-oligosaccharides), AROS (arabi-nooligosaccharides). As an alternative to these prebiotics, GroBiotic®-A, a commercial prebiotic consisting of a mixture of partially autolyzed brewer's yeast, dried fermentation products, and milk components, has seen increased use in recent years (González-Félix et al., 2018; Li & Gatlin, 2004). Although positive results have been obtained in wide variety fish species, at different trial periods and supplemented levels, studies on the effectiveness of GroBiotic®-A in crustaceans are almost non-existent. To our knowledge, only Li et al., (2009) have a study on white legs (*Litopenaeus vannamei*). To the best of our knowledge, this study will be the first attempt to apply GroBiotic®-A to an important shrimp species, green tiger shrimp (*P. semisulcatus)*.

*P. semisulcatus*, a species of Indo-Pacific origin, passed into the Mediterranean via the Suez canal and began to spread in the Eastern Mediterranean coasts and the Gulf of Iskenderun (Kumlu et al., 2000). Due to the fact that this region is suitable in terms of food and living environment for *P. semisulcatus*, a dense population has formed here, which has allowed widespread hunting (Aktaş & Kumlu, 1999). The fact that it is resistant to fluctuations in environmental factors, shows good growth, price and high market demand shows that this shrimp species will have a suitable potential for aquaculture in the Eastern Mediterranean coasts of Turkey (Aktaş & Kumlu, 1999; Aktaş et al., 2014). Although some trial studies have been carried out for its cultivation, these have not been carried out on a large scale (Kaya et al., 2020). Nutrition in the larval period, cannibalism and survival rates emerge as the most important obstacles in breeding. Therefore, in this study, the effects of adding GroBiotic®-A, which has positive effects on other aquatic organisms, to green tiger shrimp *P. semisulcatus* feeds on growth and survival rates were investigated.

## MATERIAL AND METHOD

***Experimental animals and rearing conditions:*** In this study, shrimps hatched in the fish breeding and rearing unit of İskenderun Technical University/Faculty of Marine Sciences and Technology were used. The shrimps were kept in earthen ponds for a certain period of time and then transferred to the tanks where the experiment would take place. A total of 120 shrimp weighing 1.65 g were stocked as 10 individuals in 1 $m^3$ cylindrical fiberglass tanks (Figure 1) by using a randomized experimental design. After a one-week adaptation period, the shrimp was fed and started to experiment. During the experiment, dissolved oxygen (DO, mg/L), temperature (ºC) and salinity (ppt) were measured daily with an oxygen meter (YSI, Model Y85). DO, water temperature and salinity were determined as 6.45±0.43mg/L, 27.75±1.18°C and 38.5 ppt, respectively. The experiment lasted 45 days.

**Experimental Design and Preparation of Experimental Diet:** Experimental feed was prepared by adding GroBiotic®-A to the control feed at four different levels, 0% (control), 1%, 1.5%, and 2% groups. GroBiotic®-A was kindly supplied from International Ingredient Corporation (St Louis, MO, USA). The addition of GroBiotic®-A to commercial feeds was carried out with Alphie1 with a 3-dimensional mixing feature. The prepared diets were kept in plastic containers at +4 °C until used. Each treatment group was performed in three replications. Fish were fed twice a day ad libitum during the 45-day study.

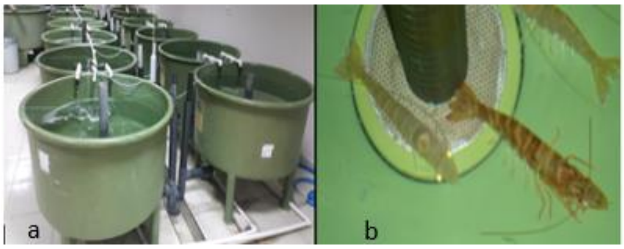


**Figure 1.** (a): a view of the tanks used in the study, (b): experimental animal.

Measuring Growth Performance and Survival Rate of Experimental Fish: Measurement of growth performance parameters was performed on day 0, day 20 and day 45 and survival was monitored daily by using the formulas below (Hoseinifar et al., 2020)

Weight gain (WG) = $W_2$ (g) - $W_1$ (g);

Specific Growth Rate (SGR) = 100 [ln $W_2$ - ln $W_1$]/T;

$W_1$ is initial weight (g), $W_2$ is final weight (g), T is time (days),

Feed Conversion Ratio (FCR) = FO/WG,

FO is feed offered (g) and WG is weight gain (g),

Survival Rate was also measured at the end of the experiment the as following formula:

Survival= $(N_f/N_0)*100$;

where $N_0$ is initial number of fish and $N_f$ is final number of fish

***Statistical Analysis:*** SPSS package program was used in the calculation of statistical analysis. Homogeneity of variances was tested before comparisons between treatment groups were performed.

One-way analysis of variance (One-way ANOVA) was used for statistical comparisons among the treatment groups, while Duncan's multiple comparison tests were used to compare means.

## RESULTS AND DISCUSSION

Although various studies have been conducted to investigate the effects of grobiotics on growth parameters such as survival rate, weight gain and specific growth rate in fish (Adel et al., 2016; Gonzalez et al., 2018), but studies in crustaceans are limited. At the end of the 45-day trial study, weight gain, feed conversion rate, specific growth rate and survival rates were determined (Table 1, Figure 1). In the present study, it was determined that the addition of GroBiotic®-A to the feed did not affect the growth parameters such as weight gain, specific growth rate and FCR of green tiger shrimp (Table 1).

Studies conducted on crustaceans and fish to date have been applied in the form of adding prebiotic substances directly to the diet, giving them together with probiotics or giving them together with live feeds such as artemia and rotifer (Naz et al., 2021). It has been shown that used MOS, the most studied prebiotic, is added to feed, it improves growth and survival of shrimps at different developmental stages. When added to the diet, MOS is found in post-larvae of green tiger shrimp (*P. semisulcatus*) (Genç et al., 2007), juvenile black tiger shrimp (*Penaeus monodon*) (Sang et al., 2014), and western king shrimp juveniles (*Penaeus latisulcatus*) showed an increase in specific growth rate (SGR) and final weight. In our current study, there was no significant difference in growth performance among the treatment groups. This situation can be attributed to factors such as starting the experiment after the shrimps used in the experiment have completed the nursery stage in open ponds, filtering of the water except for UV, size of the shrimp, the feed content, and the experiment duration. On the other hand, in a study on freshwater crayfish (*Astacus leptodactylus*) by Mazlum et al., (2011), it was found that MOS (0, 1.5, 3.0, and 4.5 g $kg^{-1}$) added to the feed at different doses was found to increase body weight. On the other hand, it was reported that there was no significant change in feed conversion ratio (FCR). Genç et al. (2007) emphasized that green tiger shrimp fed with the addition of 3 g $kg^{-1}$ MOS provided a better feed utilization rate.

On the other hand, the results of studies on the growth performance of Grobiotic-A in fish are conflicting. Previous studies indicated that GroBiotic®-A has no effect on growth performance in rainbow trout (Betiku et al., 2018; Bockus et al., 2021; Sealey et al., 2007), brown trout *Salmo trutta macrostigma*, (Sönmez et al., 2020), European sea bass *Dicentrarchus labrax*, (Yazıcı et al., 2020), red drum (Buentello et al., 2010; Rossi et al., 2017) and tilapia (Peredo et al., 2015; Vechklang et al., 2012). However, some researchers reported that Grobiotics showed an increase in growth performance in their studies with rainbow trout *Oncorhynchus mykiss* (Azari et al., 2011), red drum *Sciaenops ocellatus* (Anguiano et al., 2013), tilapia *Oreochromis niloticus*, (Zheng et al., 2011) fathead minnow *Pimephales promelas*, (Lochmann et al., 2011), great sturgeon *Huso huso* (Adel et al., 2016), starry flounder *Platichthys stellatus* (Wang et al., 2016), largemouth bass *Micropterus salmoides* (Yu et al., 2019).

Considering the survival rate; in our study, although the survival rate was high in the 2% GBA supplemented groups, it was statistically insignificant (Table 1, Figure 1). In line with our study, it was understood that the addition of 5 g kg-1 MOS did not affect the survival rate of king prawns. In contrast, the addition of MOS to the feed (2, 4, 6 and 8 g kg-1) for *L. vannamei* (Zhang et al., 2012) and 3 and 4.5 g kg-1 MOS for *P. semisulcatus* shrimp (Genç et al., 2007) reported that it increased the survival rate. An increased survival rate has been reported in white shrimp fed MOS from desiccated coconut as an alternative source of prebiotics (Rungrassamee et al., 2014). Meanwhile, Li et al., (2009) stated that there was no difference between the control group and the GroBiotic®-A supplemented groups.

Similar to the results obtained in our study, the addition of GBA did not affect the survival rate of fish in rainbow trout Betiku et al., 2018; Bockus et al., 2021), red drum (Buentello et al., 2010; Rossi et al., 2017), tilapia (Vechklang et al., 2012), starry flounder (Wang et al., 2016), goldfish (Raggi et al., 2012), *Totoaba macdonaldi* (González-Félix et al., 2018), Largemouth bass (Yu et al., 2019) and European sea bass (Yazıcı et al., 2020).

In the findings of the FCR value, the lowest value was obtained from the control group, and the highest value was obtained from the group with 1% GBA added. However, statistical differences were not observed between the control and experimental groups. As for SGR, the highest increase was observed in the group supplementation with 2% GBA, and the lowest was found in the group with 1% GBA supplementation (Table 1). Similarly, no statistical differences were observed between the control and supplement groups (P>0.05).

**Table 1.** Mean and standard deviation (±SD) of initial weight, weight gain, SGR, FCR, and survival of different levels of GroBiotic®-A on growth performance of green tiger shrimp.

| Parameters | Control | 1% GBA | 1.5% GBA | 2% GBA |
|---|---|---|---|---|
| Initial body weight (g) | 1.65±0.07 | 1.64±0.14 | 1.64±0.08 | 1.63±0.06 |
| Final body weight (g) | 3.48±0.11 | 3.26±0.19 | 3.59±0.15 | 3.84±0.2 |
| Weight gain (g) | 1.83±0.12 | 1.62±0.25 | 1.95±0.51 | 2.21±0.28 |
| Feed conversion ratio (FCR %) | 2.16±0.25 | 2.63±0.09 | 2.31±0.63 | 2.24±0.71 |
| Specific growth rate (SGR %) | 0.016±0.001 | 0.015±0.002 | 0.017±0.003 | 0.019±0.002 |
| Survival rate (SR %) | 60.00±10.0 | 60.00±10.0 | 56.66±15.27 | 73.33±5.77 |

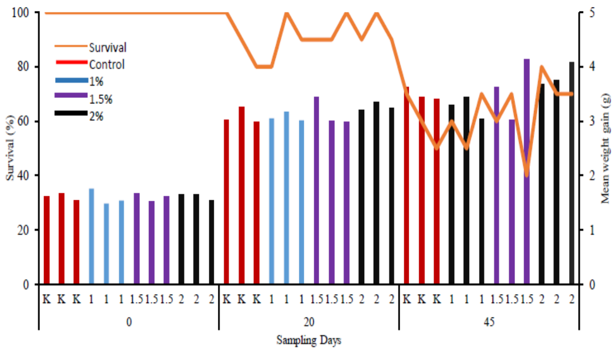


**Figure 2.** Mean weight gain and survival rate on different sample days of green tiger shrimp fed different levels of GroBiotic®-A.

## CONCLUSION

In this preliminary study, it was observed that GBA showed a statistically insignificant increasing trend in growth performance and survival rate. The best results were observed in the 2% GBA supplemented groups. In the future, before recommending the sectoral use of GroBiotic®-A, it would be useful to investigate the effects of shrimps of different sizes on immune response, digestive enzyme activity and disease resistance at different feeding periods.

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