Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. ISSN 1110 – 6131 Vol. 29(4): 2003 – 2016 (2025) www.ejabf.journals.ekb.eg



Evaluation of the Effects of Extracellular Polymeric Substance (EPS) Produced from *Lactobacillus plantarum* on Growth Performance and Survival Rate of Ricefield Eel (*Monopterus albus*) Fingerlings

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ARTICLE INFO

Article History:

Received: May 14, 2025 Accepted: July 16, 2025 Online: July 31, 2025

Keywords:

Growth performance, Edwardsiella tarda, Lactobacillus plantarum, Monopterus albus, Pathogenic bacteria

ABSTRACT

The experiment was conducted by supplementing EPS derived from Lactobacillus plantarum into the feed of ricefield eel fingerlings at concentrations of 0 (control), 4, 6, 8, and 10g/kg. After continuous feeding for two and four weeks, 90 ricefield eel fingerlings from each treatment group were challenged with the pathogenic bacterium Edwardsiella tarda. The results indicated that the optimal EPS supplementation was 8g/kg of feed for at least 2 consecutive weeks or 6g/kg for at least 4 consecutive weeks. At 8g/ kg over 2 weeks, the survival rate reached 93.3%, the specific growth rate (SGR) was 2.81%/day, and the feed conversion ratio (FCR) was 2.35. The relative percent survival (RPS) reached 84.6%, which was significantly higher compared to groups receiving lower EPS levels. Furthermore, prolonged supplementation for four weeks at a dose of 6g/kg also produced effective results. The survival rate was 93.3%, SGR reached 2.24%/day, FCR was 2.37, and RPS achieved 77.8%, all are significantly different from lowerdose treatments. In conclusion, EPS produced from Lactobacillus plantarum can be effectively supplemented into the feed of ricefield eel fingerlings at 8g/kg for at least 2 weeks, or 6g/kg for at least 4 weeks, to achieve optimal growth performance, feed efficiency, survival rate, and disease resistance (RPS).

INTRODUCTION

Ricefield eel (*Monopterus albus*) is a freshwater species that is not only economically important but also significant for the global aquaculture industry (**Cheng et al., 2021**). Consequently, the farming of *M. albus* has developed rapidly; however, growth-related issues in ricefield eels remain a major obstacle (**Yang et al., 2018**). According to a survey by **Quyen (2020)**, diseases affecting eels during the seed production process pose the







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greatest challenge, accounting for 66.7%, while 60% of households report a low survival rate from fry to fingerling stage, ranging from 50 to 60%.

Research findings indicate that ricefield eels are highly susceptible to bacterial infections at the seed stage. **Hang (2012)** identified five groups of bacteria in diseased eel samples: *Aeromonas hydrophila*, *Aeromonas* spp., *Edwardsiella tarda*, *Lactococcus* spp., and *Streptobacillus* spp. In addition, **Oanh (2012)** identified *A. hydrophila* as the primary causative agent of hemorrhagic disease in freshwater eels. More recently, a study attributed the same disease, characterized by ulcers on the ventral surface, to *E. tarda* (**Hang, 2024**). *E. tarda* is a common pathogen in aquaculture species and has been responsible for significant economic losses in the industry worldwide (**Goh** *et al.*, **2023; Adeshina** *et al.*, **2024**). According to **Sherif** *et al.* (**2020**), *E. tarda* causes high mortality rates in fish under aquaculture conditions.

Studies have demonstrated that supplementing eel feed with exopolysaccharides (EPSs) can positively influence growth, feed conversion, gut microbiota, and resistance to pathogenic bacteria while enhancing immunity (Gibson et al., 2005). EPSs not only promote favorable immune responses in fish and mollusks but also show potential to enhance the efficiency and sustainability of aquaculture production (Ringø et al., 2010).

Moreover, *Lactobacillus* bacteria can produce lactic acid and other compounds that create an unfavorable environment for harmful microorganisms in the digestive tract (**Anjana & Tiwari, 2022; Iryos** *et al.*, **2025**). They can also produce bacteriocins—proteins that kill harmful bacteria by forming membrane-permeating channels. *Lactobacillus plantarum*, a probiotic with immunomodulatory properties, has been used to boost disease resistance in the Nile tilapia (*Oreochromis niloticus*) (**Sherif** *et al.*, **2020**) and is known to alleviate diarrhea and enhance intestinal motility.

Some studies have investigated the use of immune-stimulating products to inhibit pathogenic bacteria in fish and shrimp. For instance, **Thanh Nguyen Van (2012)** explored the application of bacteriocins derived from *L. plantarum* in treating red spot disease caused by *A. hydrophila* and white spots on internal organs caused by *E. ictaluri* in pangasius. The results demonstrated that *Lactobacillus* could inhibit both pathogens effectively. Similarly, **Dung (2017)** found that eight strains of *Lactobacillus* showed antagonistic activity against *Vibrio parahaemolyticus*.

Based on previous studies investigating substances such as β -glucan, inulin, MOS, FOS, and synbiotics in species like the giant tiger shrimp, white leg shrimp, and pangasius (**Zhang** *et al.*, **2012**; **Han, 2018**; **Hang, 2020**; **Zhou** *et al.*, **2020**; **Thanh, 2021**), this research was conducted to evaluate the supplementation of EPSs from *Lactobacillus plantarum* in feed to improve the growth performance and survival rate of ricefield eel fingerlings.

MATERIALS AND METHODS

Experimental animal and conditions

This experiment was conducted at the experimental farm of An Giang University – Vietnam National University Ho Chi Minh City, located in Long Xuyen City, An Giang Province, Vietnam. Ricefield eel (*Monopterus albus*) fingerlings were sourced from a local hatchery in An Giang Province. The eels were transported to the experimental site in 0.5 m³ plastic containers with proper aeration. Upon arrival, all fingerlings underwent a prophylactic dip in a 3% NaCl solution for 5 minutes, followed by a two-week acclimatization period under laboratory conditions.

- Ricefield eel fingerlings were selected for uniform size, with an average initial weight of 2.1 g per fish.
- EPS was produced from *Lactobacillus plantarum*, based on a previous research product developed at An Giang University.
- Edwardsiella tarda strain T4 was stored at -80 °C in the laboratory of An Giang University. The strain had a determined LD₅₀ of 2.1 × 10⁴ CFU/mL (**Hang, 2024**).

Experimental design and management

The experiment followed a completely randomized design, including five treatments with different EPS supplementation levels: 0 (control), 4, 6, 8, and 10g/ kg of feed. The stocking density was 250 fingerlings per tank (160 liters/tank).

The eels were fed twice daily with a feeding rate of 5–7% of body weight per day. EPSs were dissolved in water, sprayed onto the feed, and then mixed thoroughly before feeding.

Uneaten feed was removed by siphoning approximately one hour after feeding. Water in each tank was exchanged twice daily, about one hour before feeding.

After 2 and 4 weeks of continuous feeding, 90 ricefield eels from each treatment group were challenged with the pathogen *Edwardsiella tarda* at the previously determined LD₅₀ dose. The eels were monitored for 14 days post-infection, and survival rates were recorded to assess the effectiveness of EPS supplementation in disease prevention for eel fingerlings.

Environmental factors—including dissolved oxygen (DO), pH, temperature, ammonia (NH₃/NH₄⁺), and nitrite (N-NO₂⁻)—were monitored throughout the experiment (**Boyd & Pillai, 1985**).

Measurements and Calculations

All ricefield eels were weighed and measured before and after the experiment. The following growth and health parameters were calculated:

- Specific Growth Rate (SGR)
- Weight Gain (WG)
- Daily Weight Gain (DWG)
- Feed Conversion Ratio (FCR)
- Survival Rate (SR)

SGR (%/day) = [(ln final Wt – ln initial Wt)/days] x 100

DWG (g/day) = (Final Wt - Initial Wt)/days

FCR = Total feed intake (g)/Total wet weight gain (g)

SR(%) = (Total number of fish harvest/Total number of fishes cultured) x 100

RPS (%) = [1 - (% dead eels in the treatment used EPS /% dead eels in control treatment)] x 100 (Ellis 1988).

Statistical analysis

Statistical analyses were conducted using the general linear model procedure (GLM) of the Minitab 16.0 software. Means and standard errors were calculated. One-way ANOVA and Duncan tests were used to compare means between treatment groups.

RESULTS AND DISCUSSION

Water quality

Results of monitoring water environment factors in the experiment showed that environmental factors such as temperature, dissolved oxygen (DO), pH, NH₃-/NH₄⁺ and NO₂⁻ were measured by Sera test kit. The results showed that these environmental factors were always within the allowable range and were suitable for the development of ricefield eel fingerlings. Water temperature ranged from 25.9 to 30.6°C; dissolved oxygen (DO) content ranged from 4.8 to 6.2mg/ L; and pH ranged from 7.58 to 8.03. Ammonia (NH₃/NH₄⁺) levels during the eel farming process ranged from 0.015 to 0.077mg/ L, which is within the allowable limit of < 1mg/ L. Nitrite (NO₂⁻) levels ranged from 0.001 to 0.013mg/ L, also within the acceptable limit of < 0.5mg/ L. All environmental parameters remained within suitable ranges for the healthy development of ricefield eel fingerlings (**Boyd & Pillai, 1985**).



Fig. 1. Ricefield eel fingerlings using in experiment

The effect of EPS produced from Lactobacillus plantarum on the survival rate of fingerling eels

The results of the test on the effect of EPS on the survival rate of eel larvae infected with pathogenic bacteria E. tarda at an LD₅₀ dose of 2.1x10⁴ CFU/mL are shown in Fig. (2) and Table (1).

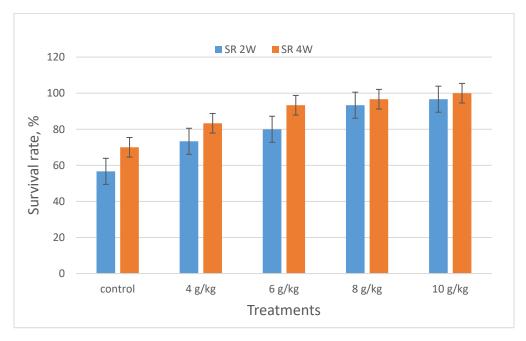


Fig. 2. The survival rate of ricefield eel fingerlings when challenged with pathogen after using EPS two and four weeks

The results indicated that the EPS supplementation treatments all gave different results compared to the control treatment (Table 1). These results demonstrate that supplementing eel fingerling feed with EPS significantly increased the survival rate—from 73.33% in the control group to 100% in the treated group—and the difference was statistically significant. This provides promising initial evidence for the effectiveness of EPS in preventing bacterial infections caused by *Edwardsiella tarda* in the ricefield eel fingerlings. Notably, no prior studies have reported the use of this specific EPS in fish; previous research has only evaluated its effects on immune responses in white-leg shrimp and giant tiger prawns. Therefore, this finding opens a new avenue for the application of EPS in disease prevention for aquaculture species, particularly in fish and eel larvae.

Table 1. The survival rate of ricefield eel fingerlings when challenged with pathogen after using EPS two and four weeks

Treatment	SR (%) at 2W	SR (%) 4W	
Control	56.7 ^d	70.0°	
4g/ kg	73.3°	83.3 ^b	
6g/ kg	80.0^{bc}	93.3 ^{ab}	
8g/ kg	93.3 ^{ab}	96.7ª	
10g/ kg	96.7 ^a	100^{a}	
P-value	0.001	0.001	
SE Mean	5.77	5.77	

SR (%) 2W: survival rate of eel fingerlings after 2 weeks; SR (%) 4W: survival rate of eel fingerlings after 4 weeks; a,b Means with different superscripts within fish species within columns are different at P<0.05.

The results in Table (1) show that the survival rate of eel fingerlings increased progressively with higher EPS supplementation levels, ranging from 4 g/kg to 10 g/kg, and also improved with longer supplementation duration—from 2 weeks to 4 weeks. Statistical analysis revealed that continuous supplementation of 6 to 10g/kg for 4 weeks resulted in significantly higher survival rates, ranging from 93.33 to 100%, compared to the other treatments. These findings suggest that supplementing over 8g/kg of EPS for 2 weeks or over 6g/kg for 4 weeks is effective in enhancing the survival rate of ricefield eel fingerlings.

Numerous studies have demonstrated that the inclusion of immune-stimulating agents such as EPSs can improve survival in aquaculture species. For instance, supplementing 1.5–2g/kg of alpha-lipoic acid (ALA) in the diet of catfish infected with *E. tarda* significantly reduced mortality to 3.3%, compared to 86.7% in the control group

(Adeshina *et al.*, 2024). Similarly, the administration of *Lactobacillus plantarum* at a concentration of 10¹² cells/kg of feed led to a notable recovery in infected fish, achieving a survival rate of 56.7%.

In another study, adding 1% inulin biweekly to catfish feed improved growth, immune function, and resistance to *E. ictaluri* (**Hang & Phuong, 2020**). Specifically, feeding pangasius with 1% inulin for 28 days resulted in a significantly lower mortality rate of 42.67% after *E. ictaluri* infection (**Han, 2018**). Compared to these studies, the present research achieved higher survival rates for eel larvae, reaching between 96.67 and 100% after just 2 and 4 weeks of EPS supplementation.

Moreover, the survival rates observed in this study are comparable to those reported in another EPS trial, where white-leg shrimp fed with 8g/ kg of EPS for 40 days achieved a 97.3% survival rate—significantly higher than the 65.4% observed in the control group (**Thanh** *et al.*, 2021). When compared with other EPS-related studies in aquatic species, the survival rates of ricefield eel fingerlings in this study were similarly high, if not higher.

For example, carp fed with 0.2% mannan oligosaccharides (MOS) achieved a survival rate of 98.08%, compared to 96.41% in the control group (**Staykov** *et al.*, **2005**). Likewise, supplementing 0.6% MOS into carp feed significantly reduced mortality from 50 to 16.7% (**Culjak** *et al.*, **2006**). Several other studies also confirm that immunostimulants like EPSs can promote growth, enhance immune responses, and increase beneficial intestinal microflora (**Chen** *et al.*, **2020**; **Zhou** *et al.*, **2020**).

Effect of EPS on the growth performance of ricefield eel fingerlings

The impact of EPS supplementation on the growth performance of eel fingerlings is summarized in Table (2). The results indicate that supplementation with EPS at levels ranging from 4 g/kg to 10 g/kg significantly improved weight gain (P < 0.05) compared to the control group, in both 2-week and 4-week continuous feeding periods.

As shown in Table (2), the highest final weight was recorded in the group receiving 8 g/kg of feed, reaching 4.14 g—an increase of 2.01 g from the initial weight. This treatment showed significantly better performance than treatments receiving less than 4 g/kg EPS. These findings suggest that 8 g/kg of EPS supplementation is the most effective dose for promoting weight gain in ricefield eel fingerlings.

	Treatments			P-	SE		
	control	4 g/kg	6 g/kg	8 g/kg	10 g/kg	value	Mean
W0 (g)	2.11 ^a	2.11 ^a	2.12 ^a	2.13 ^a	2.12 ^a	0.847	0.04
Wc (g)	3.02^{c}	3.64 ^b	3.97^{ab}	4.14 ^a	4.13 ^a	0.001	0.02
WG 2W (g)	$0.28^{\rm c}$	0.74 ^b	0.83 ^b	1.02 ^a	0.99^{a}	0.001	0.04
WG 4W (g)	$0.86^{\rm c}$	1.53 ^b	1.85 ^{ab}	2.01 ^a	2.01 ^a	0.001	0.05
DWG 2W (g/day)	0.02^{c}	0.05 ^b	0.06^{b}	0.07^{a}	0.07^{a}	0.001	0.03
DWG 4W (g/day)	0.02^{c}	0.05 ^b	0.07 ^{ab}	0.08^{a}	0.07^{a}	0.001	0.02
SGR 2W (%/day)	0.90^{c}	2.14 ^b	2.35 ^b	2.81 ^a	2.74 ^a	0.001	0.12

0.001

0.08

 2.38^{a}

Table 2. The effect of EPS on the growth performance of ricefield eel fingerlings

2.24ab

 2.38^{a}

 1.15^{c}

SGR 4W

(%/day)

 $1.92^{\rm b}$

At the same time, the results for specific growth rate (SGR) (Fig. 3) and daily weight gain (DWG) after 4 weeks were higher than those recorded after 2 weeks, with significant differences observed between the treatment groups and the control (P< 0.05) (Table 2). The data also indicated that supplementation with 8g/kg of feed for 4 continuous weeks resulted in the highest growth performance, although the differences were not statistically significant when compared to the 6 and 10g/kg treatments.

These findings align with previous research on ricefield eels, such as the study by **Hu** *et al.* (2022), which reported significantly increased weight gain at a methionine supplementation level of 6g/ kg of feed. Furthermore, the DWG and SGR values observed in this study were comparable to—and in some cases higher than—those reported in other nutritional studies. For instance, **Hang and Phuong** (2020) found that supplementing 1% inulin over 8 weeks resulted in an absolute growth rate (DWG) of 0.70g/ day and a relative growth rate (SGR) of 1.27%/day. The growth rates achieved in the present study exceeded

 $^{^{}a,b}$ Means with different superscripts within fish species within rows are different at P<0.05

W0 and Wc: initial and final weight of eel

WG 2W and WG 4W: weight gain of eel after 2 weeks and 4 weeks of experiment.

DWG 2W and DWG 4W: Daily weight gain of eel after 2 weeks and 4 weeks of experiment

SGR 2W and SGR 4W: Specific growth rate of eel after 2 weeks and 4 weeks of experiment.

these values, further highlighting the effectiveness of EPS supplementation in enhancing the growth performance of ricefield eel fingerlings.

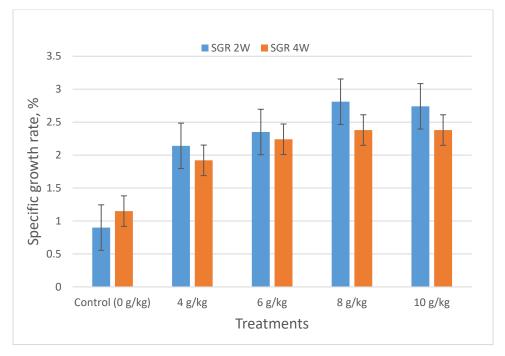


Fig. 3. The effect of EPS on the specific growth rate (SGR) of ricefiels eel fingerlings

In addition to research on EPS supplementation, studies involving other feed additives have also received considerable attention. Notably, a study supplementing a mixture of probiotics (0.4g/ kg of feed) and sodium citrate (7.5g/ kg of feed), as well as a treatment using only probiotics (0.8g/ kg of feed), demonstrated improved growth, increased total bacterial populations, and a reduced number of *Vibrio* in the digestive tract of *Monopterus albus* after 30 days of feeding (**Toi** et al., 2020). Similarly, supplementation with the probiotic *Lactobacillus acidophilus* at a density of 3×10^7 CFU/g for 12 weeks in the diet of catfish fry resulted in an SGR of 4.17% (**Al-Dohail** et al., 2009).

Another study on vitamin C supplementation in fingerling eel diets found that a dosage of 140 mg/kg feed resulted in the highest growth rate of 0.199 g/fish and a survival rate of 97%, both significantly higher than the control (**Anh, 2021**).

Thus, the weight gain and growth rate results of the present study are comparable to, and in some cases higher than, those reported in previous research. Supplementing 8g/kg of EPS for at least 2 weeks was found to be the most suitable level for enhancing the weight gain and growth performance of fingerling eels.

Feed conversion ratio (FCR)

The feed conversion ratio (FCR) of ricefield eel fingerlings after 2 and 4 weeks of continuous feeding is presented in Table (3). The results showed that all EPS-supplemented treatments had significantly lower FCRs compared to the control group (P< 0.05). Among them, the treatment with 8g/ kg EPS supplementation yielded the lowest FCRs at both time points—2.35 at 2 weeks and 2.30 at 4 weeks—which were significantly different from treatments with lower EPS levels.

Therefore, supplementation with 8g/kg of EPS was found to be the most effective in improving feed utilization in this experiment. These findings align with prior studies on ricefield eels using industrial feed, which reported an FCR of 2.92 (**Tuyen** *et al.*, **2015**), and studies combining industrial feed with trash fish, which found FCR values ranging from 2.33 to 3.35 (**Son**, **2017**). However, the FCR values observed in this study are lower than those reported by **Toi** *et al.* (**2020**), who recorded an FCR of 3.5 when supplementing 0.8 g/kg of probiotics.

In contrast, **Al-Dohail** *et al.* (2009) found that catfish fingerlings fed with L. *acidophilus* at 3×10^7 CFU/g for 12 weeks achieved an FCR as low as 1. Additionally, a study by **Hu** *et al.* (2020) reported an FCR of 1.2 in eels, while **Hang and Phuong** (2020) noted an FCR of 1.42 when supplementing 1% inulin in the catfish diets.

Although the FCR in this study is slightly higher than in some of these studies, it still represents a favorable efficiency for eel culture. Overall, the results indicate that EPS supplementation at a dose of 8 g/kg of feed is optimal for enhancing feed conversion efficiency in ricefield eel fingerlings.

Table 3. The feed conversion ratio of ricefield eels fingerling upon supplementing EPS in feed

Treatment	FCR (2 W)	FCR (4 W)
Control	2.57 ^a	2.49 ^a
4g/ kg	2.48 ^b	2.42 ^b
6g/ kg	2.42°	2.37°
8g/ kg	2.35 ^d	2.30^{d}
10g/ kg	2.38 ^{cd}	2.33 ^d
P-value	0.000	0.000
SE Mean	0.015	0.012

^{a,b} Means with different superscripts within fish species within columns are different at P<0.05

FCR 2W and FCR 4W: Feed conversion ratio of eel after 2 weeks and 4 weeks of experiment.

The efficiency of using EPS through the relative percentage survival (RPS)

In addition, the results for EPS utilization efficiency indicated that supplementation with 6–10g/kg of feed achieved a relative percent survival (RPS) ranging from 61.54 to 92.31% after 2 weeks, and from 77.78 to 100% after 4 weeks (Table 4). These results demonstrate that RPS values above 50% are considered highly effective for disease prevention in fish and are recommended for use (Amend, 1981).

Furthermore, the RPS results presented in Table (5) show that continuous EPS supplementation for 4 weeks enhanced utilization efficiency compared to 2 weeks of supplementation, with the highest RPS (100%) achieved at a dose of 10 g/kg of feed. Therefore, based on the findings of this experiment, supplementing at least 6 g/kg of EPS for a duration of 2 weeks can be considered optimal for achieving effective disease prevention in ricefield eel fingerlings.

Table 4. The efficiency of using EPS through the relative percentage survival (RPS)

Treatment	RPS (%) (2 W)	RPS (%) (4 W)	
4g/ kg	38.46 ^b	44.44 ^b	
6g/ kg	61.54 ^{ab}	77.78 ^{ab}	
8g/ kg	84.62 ^a	88.89^{a}	

10g/ kg	92.31 ^a	100.0 ^a
P-value	0.004	0.017
SE Mean	7.69	9.62

^{a,b} Means with different superscripts within fish species within columns are different at P<0.05 RPS (%) 2W and 4W: relative percentage survival of eel after 2 weeks and 4 weeks of experiment.

CONCLUSION

- The optimal EPS supplementation level was 6 to 8g/kg of feed, administered for 2 to 4 consecutive weeks, respectively, to achieve the best survival rate and growth performance in ricefield eel fingerlings.
- The lowest feed conversion ratio (FCR) was observed in treatment groups supplemented with at least 8g/ kg of EPS.
- The Relative Percent Survival (RPS) values in EPS-supplemented groups were significantly higher than in the control group, indicating strong disease resistance benefits.

ACKNOWLEDGEMENTS

This research was funded by Vietnam National University HoChiMinh City (VNU-HCM) under grant number C2023-16-07.

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