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Supplementation of Commercial Probiotic *Lactobacillus casei* in Rearing Early-Stage Silver Therapon (*Leiopotherapon plumbeus*) Larvae: Effects on Growth, Body Composition, and Stress Tolerance

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Supplementation of probiotics in live feeds and rearing water may improve the growth, survival, and resistance of fish larvae. This study examined the beneficial effect of a commercial probiotic bacteria *Lactobacillus casei* on the growth, body composition, and stress resistance in early-stage silver therapon (*Leiopotherapon plumbeus*) larvae. Larvae at 12 d post-hatch (DPH) (9.76 ± 1.32 mm total length [TL] and 13.48 ± 4.80 mg body weight [BW]) were reared in triplicate groups of 15 larvae per tank from 12 – 40 DPH (Phase 1) and 41 – 96 DPH (Phase 2). From 12 – 40 DPH, larvae were fed (I) unenriched *Artemia* nauplii (no-PB or control group); (II) probiotic-enriched *Artemia* nauplii (PB); and on (III) probiotic-enriched *Artemia* nauplii and rearing water (PB + W). The same feeding regime was used from 41 – 96 DPH except that the larvae were co-fed *Artemia* nauplii and microparticulate diet (MPD). Mean survival rates at 40 (97.78% – 100%) and 96 DPH (86.67% – 91.11%) did not show significant differences among treatments. However, except for body depth (BD) and Fulton's condition factor (*K*), mean TL, BW, length increment (LI), and length- (SGRL) and weight-specific growth rates (SGRW) of PB + W-fed larvae were significantly higher than PB-fed and no-PB-fed larvae at 40 DPH. While these parameters were similar among treatment groups at 96 DPH, slightly higher values for BW and SGRW were noted for the PB + W group, as supported by the higher proportion of *L. casei* to total microbiota in the intestines of early-stage larvae. Whole body protein and lipid contents were highest in the PB + W and PB groups, respectively, but did not differ from the no-PB group. Survival rates (93.33% – 100%) after 18 h exposure to 30 ppt did not show significant differences. However, recovery rates after 15 min of air exposure of early stage larvae in the PB and PB + W groups (93.33%) were significantly higher than those fed unenriched *Artemia* (66.67%). These results indicate that supplementation of probiotic bacteria *L. casei* in live feed and rearing water may support growth of early-stage larvae of silver therapon and its resistance to stress conditions during culture.

Keywords: growth, hypoxia, *Lactobacillus casei*, *Leiopotherapon plumbeus*, salinity stress

INTRODUCTION

The proliferation of bacteria and other pathogens may limit the production of larvae in the hatchery. Live feed organisms such as the brine shrimp *Artemia* nauplii have been reported to contain high bacterial load and, as such, could be a potential source of diseases when offered to the larvae. Bioencapsulation or probiotic administration, either via live feeds or added to the rearing water, has been proposed to reduce the bacterial load in the rearing system (Gatesoupe 1999; Skjermo and Vadstein 1999; Yanes-Roca et al. 2020). Probiotics, considered as live microbial feed supplements, improve the intestinal microbial balance of the host animal (Fuller 1989; Hill et al. 2014). It also helps to stimulate the immune system by competing with pathogens for nutrients (Gatesoupe 1999; Vine et al. 2004; Balcázar et al. 2006), thus preventing the successive colonization of pathogenic bacteria in fish larvae (Vanbelle et al. 1990). Among the probiotic bacteria used in fish hatcheries, gram-positive lactic acid bacteria (LAB) have been shown to improve growth, survival, and resistance for a wide variety of fish species such as porthole livebearer (*Poecilopsis gracilis*) juvenile (Hernandez et al. 2010), grouper (*Epinephelus coioides*) (Sun et al. 2010), pikeperch (*Sander luciopera*) (Ljubobratovic et al. 2017; Ljubobratovic et al. 2020; Yanes-Roca et al. 2020), and seabream (*Sparus aurata*) larvae (Suzer et al. 2008). Also considered as the most abundant probiotic microorganisms, LAB have been proven to increase resilience to stress conditions, provide antimicrobial effects, and stimulate the fish immune system (Ringø and Birkbeck 1999; Ljungh and Wadström 2006; Ringø et al. 2018).

Regarded as one of the tastiest native freshwater fish species caught in Laguna de Bay, Philippines, the silver therapon *Leiopotherapon plumbeus* comprised the artisanal fishery in lakeside communities. Although the breeding and larval rearing protocols are well-established for this fish species (Aya and Garcia 2020; Aya et al. 2022), survival in the early larval stages still needs to be improved. In an earlier study (Aya et al. 2021), early juveniles of silver therapon larvae co-fed unenriched *Artemia* nauplii and commercial prawn diet from 36 to 95 d post-hatch (DPH) had survival, length- and weight-based specific growth rate of 97%, 0.95%, and 2.85%/day, respectively. This fish species is very sensitive to handling stress and means to increase their resilience to stress conditions are necessary. However, no information related to the resistance of early-stage silver therapon larvae to stress conditions has been reported. It is hypothesized that supplementation of a commercial probiotic such as *Lactobacillus casei* from the commercial product Yakult® via *Artemia* nauplii and rearing water may improve the growth and resistance of early-stage silver therapon larvae to stress during culture. In addition, the price of Yakult® is reasonable, making *L. casei* an affordable option in aquaculture production (Hernandez et al. 2010).

The use of dietary probiotics during early feeding stages to improve growth and survival has been considered as a sound strategy for sustainable development of aquaculture (Verschuere et al. 2000). Therefore, this study examined whether supplementation of a commercial probiotic *Lactobacillus casei* in *Artemia* nauplii and rearing water has a beneficial effect on growth, body composition, and resistance to stress in early larval stage of silver therapon.

MATERIALS AND METHODS

Fish Larvae

Early-stage larvae (n = 135) used in this study came from eggs of hatchery-reared silver therapon broodstock following the hormone-induced spawning protocol of Aya and Garcia (2020) and Aya et al. (2022). Eggs were incubated and hatched at 25ºC – 27°C in plastic basins provided with mild aeration. Newly-hatched larvae were initially stocked at 500 larvae each in two 4-m^3 outdoor tanks previously filled with lake water up to 0.5 m and fed on natural food organisms present in lake water (Aya et al. 2019). Early-stage larvae (11 DPH) were harvested for stocking in 10-L capacity rectangular plastic tanks (effective water volume of 3 L). During stocking, initial body weight (BW), total length (TL), and body depth (BD) were measured and recorded (to the nearest ± 0.01 g and ± 0.01 mm, respectively). Trials were conducted at the Binangonan Freshwater Station of the Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC/AQD).

Experimental Treatments

Larvae of silver therapon (15 per tank) were reared in two phases: Phase 1 (12 – 40 DPH) and Phase 2 (41 – 95 DPH). Twelve-day-old silver therapon larvae with a mean TL and BW of 9.76 ± 1.32 mm and 13.48 ± 4.80 mg, respectively were reared in three treatment groups: (I) a control group of larvae fed unenriched *Artemia* nauplii (no PB); (II) larvae fed probiotic-enriched *Artemia* nauplii (PB); and (III) larvae fed both on probiotic-enriched *Artemia* nauplii and rearing water (PB + W). Each treatment group had three replicate 10-L capacity plastic tanks filled with 3 L of ground tap water. Experimental tanks were set up in an indoor hatchery facility exposed to natural light conditions during daytime.

Live Feed Enrichment and Feeding

In Phase 1 (12 – 40 DPH), larvae in the control group were fed A*rtemia* nauplii four times daily (0900, 1100, 1400, 1600 H) with a concentration of 5 ind/mL (Fig. 1). Experimental larvae in the two probiotic-enriched groups were also fed on the same hours at equal rations. Newly-hatched *Artemia* nauplii were enriched for 1 h with Yakult® using commercial *Lactobacillus casei* cells and the fermented milk with 3.00 × 10^7 CFU/mL at a 50 mL:1 mL ratio with continuous aeration, after which the probiotic-enriched *Artemia* had 2.23 × 10^7 CFU/mL (based on plate counting using *Lactobacillus* MRS agar; Titan Biotech Ltd., Rajasthan, India). In the 3rd treatment, probiotic was supplied in the rearing water at a daily dose of 2 mL (1 mL each in the morning and afternoon feeding) (Ghosh et al. 2008). Larvae were reared for 29 d.

In Phase 2 (41 – 96 DPH), the same feeding regimes were used except that both live and microparticulate diet (MPD) were fed to the larvae. Larvae were co-fed unenriched or enriched *Artemia* nauplii at 3 ind/mL and MPD at 25% of the estimated biomass of the larvae in each tank for another 56 d (Fig. 1). To compensate for the increase in biomass across all treatments, water level in the rectangular tanks was increased to 6 L, reducing the stocking density at 3 fish/L and the daily dose of probiotic supplied in the rearing water to 1 mL (Fig. 1).

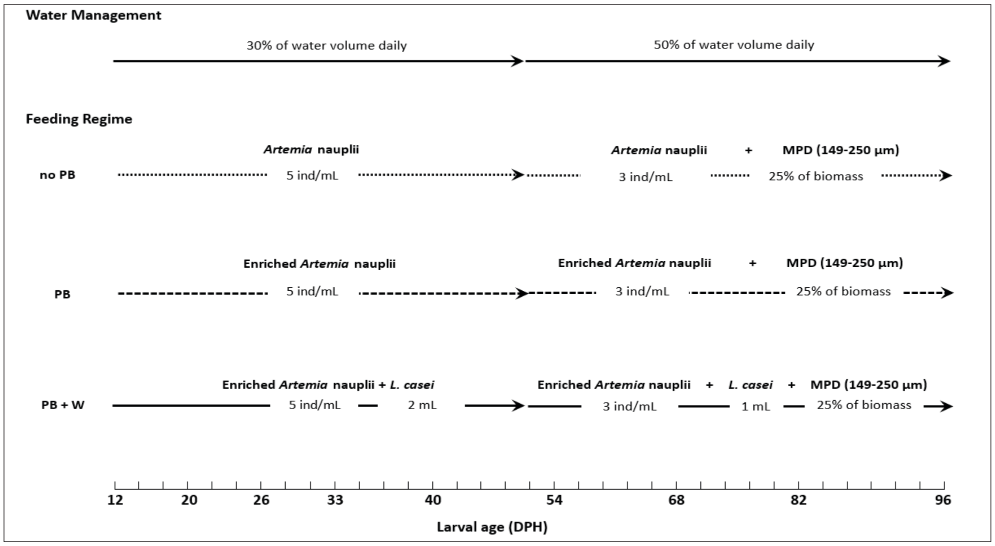

Fig. 1 Water management and feeding regimes in rearing early-stage silver therapon *Leipotherapon plumbeus* larvae.

Water Management

All tanks were cleaned before the first feeding episode at 0900 h by siphoning out waste products and scrubbing tank walls (Fig. 1). Water temperature and dissolved oxygen (DO) were recorded thrice per week using a portable water quality meter. The pH and nutrient concentrations (Total Ammonia Nitrogen [TAN], nitrite and nitrate) were measured twice per week using a pH meter and API test kits.

Sampling

Five larvae were sampled weekly in Phase 1 and biweekly in Phase 2 from each replicate tank by gently scooping them out of the tanks. TL, BD, and BW of each larva were measured with a digital caliper (± 0.01 mm) and an electronic balance (± 0.01 g), respectively and larvae returned immediately to their respective tanks. Survival was estimated by counting the number of surviving larvae in each replicate tank at 40 and 96 DPH relative to the total initial number of larvae stocked. At the end of the 85-d feeding trial, the fish were starved for 24 h before individual TL, BD, and BW measurements were taken.

Bacterial and Proximate Analyses

Two fish larvae per replicate tank (total of six larvae per treatment group) were collected for bacterial analysis. Intestines were dissected in sterile conditions, pooled due to limited samples, homogenized, and serially diluted (up to 10^{-2}) with sterilized normal saline solution (NSS). Bacterial suspension (0.01 mL) was placed onto nutrient agar plates for total plate count (TPC) and *Lactobacillus* MRS agar plates for LAB count each in triplicates. The TPC agar plates were incubated at 28°C for 24 h while LAB agar plates were incubated at 37°C for 48 h. Following incubation, colonies were counted, and TPC and LAB were calculated as the number of colony-forming units per gram of intestine (CFU/g) (Bagheri et al. 2008).

The remaining fish specimens were pooled for each treatment group and oven-dried (60°C for 24 h) for proximate (e.g. moisture, crude protein, crude lipid, and ash) analysis using the standard AOAC (2016) methods.

Salinity and Hypoxia Stress Tests

After the 85-d feeding trial, early silver therapon juveniles (n = 5 per tank or 15 per treatment) were randomly captured and transferred to a 1.5-L rectangular plastic container (n = 3), where they were exposed to a salinity of 30 ppt for 18 h. The salinity level used was based on an earlier study of Corpuz and Manliclic (2020) who found that the LD_{50} is achieved at 30 ppt. Mortalities were monitored every 30 min for the first 2 h and every hour thereafter.

An air-dive or hypoxia stress test was also conducted where five silver therapon larvae per replicate tank (or 15 per treatment group) were randomly captured, transferred in a water-filled basin, and placed in a dry fish scoop net (Sakakura et al. 1998; Yokoyama et al. 2006; Hernandez et al. 2010). After 15 min, the fish were placed in a 1.5-L rectangular plastic container (n = 3 per treatment group) and dead fish or those lacking gill movement were recorded within 3 h after being returned to the plastic container. Mean survival or recovery rate (%) from the salinity and hypoxia stress tests was calculated based on the equation (Hernandez et al. 2010):

$$\text{Recovery rate (\%)} = (N_i - N_f) / N_i \times 100$$

where N_i is the initial number of test fish and N_f as the dead fish after the salinity and hypoxia stress tests.

Statistical Analysis

Mean TL, BD, BW, percent survival, water quality parameters, TPC and LAB counts, whole body composition and recovery rate of salinity and hypoxia stress tests were compared among treatment groups by one-way analysis of variance followed by Least Significant Difference (LSD) post-hoc test at $p = 0.05$. Prior to statistical analysis, all percentage data were arc-sin transformed and data checked for normality and homogeneity of variance by Anderson-Darling and Barlett tests, respectively before analysis.

RESULTS AND DISCUSSION

Probiotic supplementation had no significant effect on survival of early-stage larvae of silver therapon at 40 and 96 DPH (Table 2). Mean survival at 40 DPH ranged from 97.78% in larvae fed probiotic-enriched (PB) and unenriched *Artemia* (no PB; control group) to 100% in larvae fed probiotic-enriched *Artemia* and rearing water (PB + W). At 96 DPH, larvae in the no PB or control group had slightly higher mean survival (91.11%) than those in the PB and PB + W groups (86.67%). Similar results have been observed in cobia *Rachycentron canadum* (Garrido-Pereira et al. 2014) and Florida pompano, and in common snook and red drum (Hauville et al. 2016) larvae fed diets enriched with commercial mix of *Bacillus* sp., suggesting probiotic supplementation did not increase larval survival. In contrast, administration of lactic acid bacteria *Pediococcus acidilactici* through the live feed and rearing water significantly improved pike perch larval survival at 21 DPH (Yanes-Roca et al. 2020). Supplementation of probiotic bacteria in live feed and rearing water has been reported to reduce the ammonia and nitrate concentrations in the rearing water (Zokaeifar et al. 2014; Elsabagh et al. 2018; Mohammadi et al. 2020; Yanes-Roca et al. 2020). Indeed, among the water quality parameters monitored in this study, nitrate (NO_3-N) concentrations were significantly lower (1.27 mg/L) when probiotic bacteria *L. casei* was supplemented in the PB + W group compared to the other treatments (3.01 – 3.02 mg/L) (Table 1). Similarly, lower concentrations of Total Ammonia Nitrogen (TAN) and nitrite (NO_2-N) were also measured in the PB + W group (0.11 and 0.06 mg/L, respectively), although the recorded values were not statistically different between treatments.

The growth performance of early-stage silver therapon larvae was significantly improved by probiotic supplementation at 40 DPH but not at the end of the trial (Table 2). Larvae in the PB + W group had significantly higher mean TL (21.83 mm), LI (12.07 mm), and BW (129.33 mg) than the control group (20.13 mm TL, 10.37 mm LI, and 94.00 mg BW), but were comparable to those in the PB group (20.03 mm TL, 10.27 mm LI, and 103.33 mg BW). Similarly, length- and weight-based specific growth rates (SGRL and SGRW) were also significantly highest in the PB + W group

Table 1. Physico-chemical parameters monitored in rearing tanks stocked with early-stage silver therapon *Leiopotherapon plumbeus* larvae and fed probiotic-enriched *Artemia* nauplii from 12 to 96 DPH.

Parameters	Feeding Regime		
	no PB	PB	PB + W
Temperature (°C)			
AM	27.38 ± 0.10	27.36 ± 0.11	27.38 ± 0.04
PM	27.75 ± 0.10	27.76 ± 0.13	27.79 ± 0.04
DO (mg/L)			
AM	6.03 ± 0.19	5.83 ± 0.16	5.88 ± 0.11
PM	7.05 ± 0.18	7.01 ± 0.23	6.99 ± 0.15
pH			
AM	8.24 ± 0.03	8.23 ± 0.04	8.24 ± 0.02
PM	8.51 ± 0.03	8.50 ± 0.05	8.51 ± 0.02
TAN (mg/L)	0.16 ± 0.05	0.17 ± 0.02	0.11 ± 0.01
NO_2-N (mg/L)	0.12 ± 0.01	0.10 ± 0.03	0.06 ± 0.03
NO_3-N (mg/L)	3.02 ± 0.50[a]	3.01 ± 0.56[a]	1.27 ± 0.16[b]

AM, morning; PM, afternoon; DO, Dissolved oxygen; TAN, Total Ammonia Nitrogen. Data are mean ± S.E. ($n = 3$). Treatment row means sharing different superscripts indicate significant difference ($p < 0.05$).

(2.77 and 7.79 %/d, respectively). Such an improvement in growth parameters was also found in earlier studies involving Porthole livebearer *Poecilopsis gracilis* juveniles (Hernandez et al. 2010), seabass (*Dicentrarchus labrax*) (Hamza et al. 2016), pikeperch (*Sander luciopera*) (Yanes-Roca et al. 2020), and Atlantic cod (*Gadus morhua*) larvae (Puvanendran et al. 2021). Improved growth is attributed, in part, to the appetite-stimulating effect of probiotic bacteria which led to increased feed utilization (Yanes-Roca et al. 2020) or due to increased levels of an insulin-like growth factor (IGF) that promote muscle growth in fish (Carnevali et al. 2006; Carnevali et al. 2017). However, BD and Fulton's condition factor (K) were not statistically different among the treatment groups (Table 2). At 96 DPH, while no significant differences in growth parameters were detected, slightly higher values for TL, LI, BD, BW, SGRL, and SGRW were observed in early-stage larvae fed probiotic-enriched *Artemia* nauplii (PB and PB + W) compared to the control group (Table 2). Similar findings were observed in a study on gilthead seabream (*Sparus aurata* L.) larvae when commercial probiotic *Bacillus* sp. was supplemented in live feed and rearing water (Ariğ et al. 2013). In comparison, the findings in this study were better than the values of the growth parameters obtained for the same species co-fed unenriched *Artemia* and commercial feeds (Aya et al. 2021), suggesting that probiotic supplementation in rearing early-stage silver therapon larvae is beneficial and necessary. In addition, the observed growth improvement in this study and other studies may be explained, in part, by the gut colonization of probiotic

Table 2. Survival, growth and condition factor of early-stage silver therapon *Leiopotherapon plumbeus* larvae fed probiotic-enriched *Artemia* nauplii from 12 to 96 DPH.

Parameters	Feeding Regime		
	no PB	PB	PB + W
Survival (%)			
40 DPH	97.78 ± 2.22	97.78 ± 2.22	100
96 DPH	91.11 ± 5.88	86.67 ± 7.70	86.67 ± 7.70
TL (mm)			
12 DPH		9.76 ± 0.59	
40 DPH	20.13 ± 0.51[a]	20.03 ± 0.36[a]	21.83 ± 0.43[b]
96 DPH	31.15 ± 0.72	32.88 ± 1.06	32.78 ± 0.67
LI (mm)			
40 DPH	10.37 ± 0.51[a]	10.27 ± 0.36[a]	12.07 ± 0.43[b]
96 DPH	21.39 ± 0.72	23.12 ± 1.06	23.02 ± 0.67
SGRL (%/d)			
40 DPH	2.49 ± 0.09[a]	2.48 ± 0.06[a]	2.77 ± 0.07[b]
96 DPH	1.36 ± 0.03	1.43 ± 0.04	1.42 ± 0.02
BD (mm)			
12 DPH		4.34 ± 0.24	
40 DPH	5.60 ± 0.09	5.77 ± 0.07	5.83 ± 0.05
96 DPH	8.59 ± 0.16	9.08 ± 0.34	9.01 ± 0.21
BW (mg)			
12 DPH		13.48 ± 2.15	
40 DPH	94.00 ± 5.03[a]	103.33 ± 9.61[a]	129.33 ± 5.21[b]
96 DPH	435.29 ± 26.97	487.94 ± 42.81	499.77 ± 12.70
SGRW (%/d)			
40 DPH	6.69 ± 0.19[a]	6.99 ± 0.31[a]	7.79 ± 0.14[b]
96 DPH	3.01 ± 0.06	3.12 ± 0.09	3.15 ± 0.03
K			
40 DPH	1.15 ± 0.05	1.28 ± 0.05	1.24 ± 0.05
96 DPH	1.44 ± 0.01	1.37 ± 0.02	1.42 ± 0.05

TL, total length; LI, length increment; SGRL, length-based specific growth rate; BD, body depth; BW, body weight, SGRW, weight-based specific growth rate; *K*, Fulton's condition factor. Data are mean ± S.E. ($n = 3$). Treatment row means sharing different superscripts indicate significant difference ($p < 0.05$).

bacteria which enhanced the activities of digestive enzymes and improved feed digestion and assimilation (Ariğ et al. 2013). While analyses of the digestive enzyme activities were not performed in this study, the higher proportion of *L. casei* to the total microbiota in the intestinal flora of early-stage larvae fed probiotic-enriched *Artemia* (PB and PB + W; Table 3) compared to the control group suggests high colonization success of microbial cells in a suitable intestinal or gut environment (Ringø et al. 1995; Vine et al. 2006; Bagheri et al. 2008). Further studies examining the microbiota diversity should be done to examine the extent of the colonization success of probiotic bacteria in early-stage silver therapon larvae.

The slightly better growth performance of the probiotic-fed early-stage silver therapon larvae may also be explained by the nutritional benefits of the probiotic bacteria *L. casei*. Balcázar et al. (2006) noted that some probiotic strains may supplement food and their digestive tract activities may be a source of essential nutrients. Their nutritional effect is reflected in the body proximate composition of fish (Ghosh et al. 2007). In this study, whole body protein (61.33%) and lipid (7.17%) contents were highest in the PB + W and PB groups, respectively, but the differences with those of the no PB group were not significant (Table 4). Whole body moisture content was significantly higher in the no PB or control group than the PB group but did not differ with that of the PB + W group. Similar results have been observed in Nile tilapia (*Oreochromis niloticus* L.) fed diets with probiotic Biogens (El-Haroun et al. 2006). In contrast, Hernandez et al. (2010) found no significant differences in the body proximate composition of *P. gracilis* juveniles fed *L. casei*-enriched *Artemia* nauplii and the control group.

Other potential benefits of probiotic supplementation in rearing early-stage silver therapon larvae may be physiological in nature. Similar to other fish species such as the pikeperch (Yanes-Roca et al. 2020), silver therapon has low tolerance to handling stress conditions during culture (Aya and Garcia 2020). The influence of probiotic supplementation on salinity stress tolerance after 18 h exposure to 30 ppt was not significant, although early-stage larvae of silver therapon

Table 3. Relative proportion of *Lactobacillus* (LAB) to total plate count (TPC) in the intestine of early-stage silver therapon *Leiopotherapon plumbeus* larvae fed probiotic-enriched *Artemia* nauplii from 12 to 96 DPH.

Parameters	Feeding regime		
	no PB	PB	PB + W
LAB (CFU/g)	7.53×10^6	4.14×10^6	6.97×10^7
TPC (CFU/g)	1.01×10^8	9.08×10^6	1.62×10^8
%	6.98 ± 0.77[a]	45.59 ± 1.08[b]	42.75 ± 0.48[b]

Data are mean ± S.E. ($n = 2$). Treatment row means sharing different superscripts indicate significant difference ($p < 0.05$).

Table 4. Proximate composition (% dry matter) of larval feeds and early-stage silver therapon *Leiopotherapon plumbeus* larvae fed probiotic-enriched *Artemia* nauplii after 85 d of feeding.

	Larval feeds		Feeding regime		
	Enriched *Artemia*	Microparticulate Diet[b]	no PB	PB	PB + W
Moisture	13.02	8.6	12.32 ± 0.23[a]	10.42 ± 0.21[b]	11.81 ± 0.80[ab]
Crude protein	42.71	45.62	58.36 ± 2.89[ab]	51.55 ± 2.54[b]	61.33 ± 1.27[a]
Crude fat	9.46	6.91	4.74 ± 0.85[ab]	7.17 ± 0.29[a]	3.91 ± 0.87[b]
Crude ash	7.43	12.17	22.91 ± 0.52	21.34 ± 2.27	20.51 ± 0.86

[a]Data are mean ± S.E. ($n = 3$). Treatment row means sharing different superscripts indicate significant difference ($p < 0.05$).
[b]Aya et al. (2021)

in the control group had slightly higher mean survival (100%) than PB and PB + W treatment groups (93.33%) (Fig. 2a). This was not the case for the pikeperch larvae, where probiotic bacteria *P. acidilactici* addition in both live feed and rearing water significantly enhanced the larval stress tolerance after a 3-h exposure test compared to the control (Yanes-Roca et al. 2020). In addition, early larvae reared with probiotic bacteria (PB and PB + W) showed significantly higher mean recovery rates (93.33%) after 15 min of air-dive test (Fig. 2b), suggesting better tolerance to stress and larval health condition compared to those fed unenriched *Artemia* (66.67%). This confirms an earlier finding on juvenile *P. gracilis* in which the addition of *L. casei* significantly improved its tolerance to hypoxia stress (Hernandez et al. 2010).

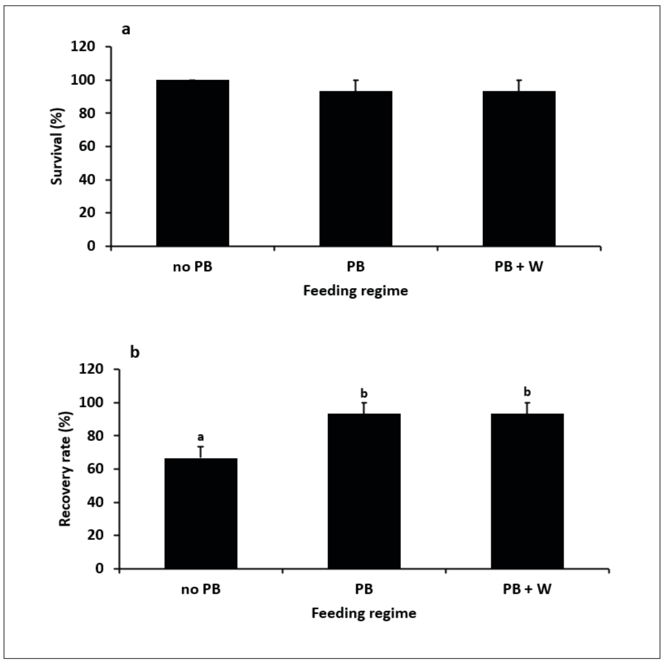

Fig. 2 Survival after salinity stress test (a) and recovery rates after air-dive test (b) of early-stage silver therapon *Leiopotherapon plumbeus* larvae fed probiotic-enriched Artemia from 12 to 96 DPH.

CONCLUSION

This study demonstrated that feeding early-stage silver therapon larvae with probiotic *Lactobacillus casei* significantly enhanced tolerance to stress condition, leading to increased robustness during nursery culture. There was no significant effect of probiotic administration on larval growth, the enhanced dietary nutrients—particularly crude protein and crude lipid levels in probiotic-enriched groups concomitant with high proportion of *L. casei* in the gut—resulted in slightly heavier early-stage larvae. Further studies on microbiota composition in the larvae during the probiotic feeding period are necessary to examine the application of *L. casei* or other probiotic bacteria in rearing silver therapon larvae.

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