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Assessment of effective microorganism (EM-1) additive on weight and length gain rate of juvenile discus (*Symphysodon* sp.)

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ABSTRACT

Aim: The study was aimed to assess the effects of a daily 1 mlL^{-1} EM-1 additive into aquarium water on the selected growth parameters, water pH and conductivity of juvenile Discus fish.

Method and materials: Prior to the five-day adaptation period, forty, 45-day-old juvenile Discus (*Symphsodon* sp.) were split into four groups: Group 1 (control) was treated with basal ration, 2% spirulina and preventive medicine (250 mg100L^{-1} metronidazole and 250 mg100L^{-1} praziquantel); and Group 2 was treated with basal ration, 2% spirulina, preventive medicine and 1 mlL^{-1} EM-1, Group 3 was treated with basal ration, 2% spirulina and 1 mlL^{-1} EM-1, and Group 4 was treated with basal ration, preventive medicine and 1 mlL^{-1}EM-1.

Results: Vertical length gain rate (VLGR) and weight gain rate (WGR) were significantly lower in Group 1 and higher in Group 3 ($p<0.05$). Based on these findings, it was determined that supplementation of EM-1 decreased pH and increased VLGR and WGR, decreased feed conversion rate in discus juveniles comparing to control group.

Conclusion: It was concluded that use of EM-1 as a probiotic in discus feed and spirulinain juvenile discus feed improved FCR, WGR and VLGR compared to the control group. EM use in planted aquariums and effects on the mating behavior may be tested before using in these conditions.

Keywords: Beneficial microorganisms, growth performance, probiotic, *Spirulina*, Water quality.

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Introduction

Discus (*Symphsodon* sp.) is a popular and expensive ornamental fish breed that originated from the Amazon River (Chong et al., 2002; Crampton, 2008). In 2007, United States exported 315 million USD worth of ornamental fish to nearly 100 countries (Monticini, 2010). This significant economic sector reached almost 5 billion USD in 2018 (Hoseinifar et al., 2023). The disease outbreak has resulted in a significant economic loss, estimated to be worth around 400 million USD in ornamental fish market (Evan & Putri, 2019). Survival rate of ornamental fish is critical for this volume of trade. Diseases that effecting survival rate of ornamental fish could be parasitic, bacterial, viral or fungal origins and most of diseases root causes are poor quality or contaminated water (Hoseinifar et al., 2023).

Antibiotics are generally used to treat diseases, and antibiotic misuse is still prevalent in aquaculture (Shao et al., 2021). There is rising concern about overuse of antibiotics in ornamental fish sector and its potential impact on drug resistance in both biotic and microorganisms that are pathogenic in these fish (Rose et al., 2013; Zhou et al., 2009). Microorganisms in marine habitats produce novel bioactive chemicals, including substances that could be utilized as antibiotic alternatives, and these microorganisms might function as feed supplements to improve overall health of aquaculture species' and water quality characteristics (Hamza & Zinjarde, 2023). Researchers are currently seeking for new feed additives and methods to reduce the use of antibiotics. Probiotics have been employed in aquaculture practices since early 1980s to control bacterial infections and improve water quality (Hoseinifar et al., 2023). Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and yeast, mixture of probiotic strains and symbiotic are widely used in aquaculture production (Wang et al., 2019). As an example such as *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Carnobacterium* sp., and yeast *Saccharomyces cerevisiae* are commonly used in fish production (Martínez Cruz et al., 2012). Microbial feed additives have a variety of beneficial effects, including increasing food conversion efficiency and rate, as well as regulating water quality parameters in ornamental fish (Hoseinifar et al., 2023). Probiotics also promote mutual affinity between bacteria and fish mucosal flora (Balcázar et al., 2006; Alak & Atamanalp, 2012). Probiotics appear to be effective alternative to antibiotics, particularly in preventative medicine (Zhou et al., 2009).

Effective Microorganisms is a technologically developed probiotic that contains Phototropic bacteria, lactic acid bacteria, actinomycetes, fermentation fungi and yeasts, and nonpathogenic beneficial microorganisms (Higa &Wididana, 1991; Higa & Parr, 1994; Szymanski & Patterson, 2003).EM has been used in fields such as food, agriculture, organic farming, animal husbandry, fisheries, environmental cleaning, bioremediation of water and medicine; in addition, some of the countries are using EM in their environmental policies (Chen et al., 2003; El Shafei & Abd Elmoteleb, 2018; Namsivayam et al., 2011; Siripornadulsil & Labteephanao, 2008; Szymanski & Patterson, 2003; Thangavelu & Veeraragavan, 2022). Secretions from photosynthetic bacteria are used in the development of other microorganisms. Previously EM-1solution matters reported as mixture of lactic acid bacteria *Lactobacillus planetarium* (1.0×10^4 CFUml^{-1}) yeast with 1.0×10^5 CFUml^{-1} *Candida utilis*, actinomycetes *Streptomyces albus* (3.0×10^3 CFUml^{-1}) and fermenting fungi *Aspergillus oryzae* (1.1×10^5 CFUml^{-1}) (Namsivayam et al., 2011). The fundamental components of the EM effect mechanism are as follows: Lactic acid is an effective sterilizer that masks harmful microorganisms and accelerates the disintegration of organic matter, antimicrobial and useful substances are synthesized by yeasts from amino acids and sugars secreted by photosynthetic bacteria for fish growth and microalgae mostly photosynthesize in order to absorb CO_2 and provide O_2 to aquatic species, whereas bacteria and fungi primarily deteriorate organic waste in sediments in order to keep the water clean (Cóndor_Golec et al., 2007; Esatu et al., 2011 and Zhou et al., 2009).

Not only probiotics, but also feed ingredients and water quality, play an important role in improving Discus fish health and growth rates. Chong et al. (2000) suggested that Discus rations must contain at least 449-501 g kg^{-1}protein. Spirulina presents protein and essential fatty acid resource, and an immune modulator agent in Discus breeding aquariums (Gutiérrez-salmeán et al., 2015). Spirulina is also used as a protein, color and immune system supplement in Discus breeding (Livengood et al., 2009 and Yeşilayer, 2008). Discus fish breeding requires a stable aquarium environment and water quality, as well as minimal stress factors and adequate feeding (Çelik et al., 2008). Main purpose of the study was to assess the effects of a daily 1 mlL^{-1} EM-1 additive into aquarium water on the selected growth parameters and water pH and conductivity of juvenile Discus fish.

Materials and Methods

Forty juvenile (45-days-old) Discus fish were divided equally four $40\times40\times50$ cm^3 aquariums that were designed in a static system described previously by (Çelik et al., 2008). Optimal space has been reported as 7.5 L for juvenile discus (Tibile et al., 2016). Fish density was eight fish L^{-1} in each aquarium. Water temperature was stabilized at 29.5±0.27°C by 200-Watt thermostats and filtered by an active carbon filter containing three-levels of sediment and sterilized by 18-Watt UV light. 25% of the water was turned over periodically every day. Nitrite level was controlled two times a day (alert limit ≤ 0.1 mgkg^{-1}) in terms of harmful levels of nitrogen and ammonium and nitrite level was never found above this value.

Following five-day adaptation period, a 28-day nutrition trail was initiated. Fish were fed five times a day with beef hearth, shrimp, garlic, frozen blood worm (*Chironomidae*), live white worm and granule discus feed in accordance with recommended doses (Sweeney, 1996) (Table 1, 2, 3). This ration was accepted as the basal ration for all groups.

Discus fish were threatened by bacterial, viral, parasitic, and fungal diseases. Therefore, diseases were important stressor factors and pose a problem for breeders. Fish owners usually use preventive medication for parasite infections by the use of anthelmintics.

Table 1. Composition of feed materials

Items (%)	Granule Discus Feed *	Beef Heart	*Spirulina***	Blood Worm	White Worm
Moisture	25.00	64.65	4.68	16.50	5.90
Crude Protein	53.00	15.90	57.50	60.00	57.50
Crude Fat	10.30	14.80	7.72	2.60	10.10
Crude Fiber	2.90	3.40	3.60	4.90	4.60
Crude Ash	8.80	1.25	6.23	16.00	5.80

*Vit. A 37.000 IU kg^{-1}, Vit.D_3 1.800 $IUkg^{-1}$, Vit. E $mgkg^{-1}$, Vit B_1 35 $mgkg^{-1}$, Vit. B_2 90 $mgkg^{-1}$, Vit C 550 $mgkg^{-1}$; **: 0,05g *Spirulina* was added in G_1, G_2, G_3 groups.

Table 2. Daily ration in the experimental period for ten fish

	Phases								
	First			Second			Third		
DFS	1.-13.	3., 7., 10.	5., 12.	14.-21.	19.	14., 17., 21.	22.-28.	24., 29.	23., 27.
First (g)	3.0 BH			4.0 BH			5.0 BH		
Second (g)	0.4 DF			0.5 DF			0.8 DF		
Third (g)	3.0 BH	4.0 BW		4.0 BH		5.0 BW	5.0 BH	6.0 BW	
Fourth (g)	0.4 DF		4.0 WW	0.5 DF	4.0 WW		0.8 DF		5.0 WW
Fifth (g)	3.0 BH			4.0 BH			5.0 BH		
Total (g)		17.8			22.0			27.6	

Abbreviations: DFS: Daily Feeding Session, BH: Frozen Beef Heart, DF: Pelleted Discus Feed, BW: Frozen Blood Worm, WW: Live White Worm.

Table 3. Composition of the rations on the feeding phases

Items (%)	Phase I *	Phase II	*Phase III*
Moisture	40.00	42.47	42.48
Crude Protein	38.21	36.23	36.23
Crude Fat	11.21	11.30	11.37
Crude Fiber	4.13	4.06	4.03
Crude Ash	6.15	5.95	5.89

*Vit. A 37.000 IU kg^{-1}, Vit.D_3 1.800 $IUkg^{-1}$, Vit. E $mgkg^{-1}$, Vit B_1 35 $mgkg^{-1}$, Vit. B_2 90 $mgkg^{-1}$, Vit C 550 $mgkg^{-1}$; 0,05g *Spirulina* was added in G_1, G_2, G_3 groups.

Preventive medicine is an inseparable part of discus breeding, and it should be linked with feeding (Giovanetti, 1991). Metronidazole (250 $mg100L^{-1}$, Flagyl, Eczacıbaşı®, Istanbul, Türkiye) and praziquantel (250 mg100 L^{-1}, Droncit, Bayer®, Istanbul, Türkiye) were administered to all groups except Group 3 as a parasite and bacterial infection preventive medicine.

EM-1 (EM Agriton® İzmir, Türkiye) was used by adding 1 mlL^{-1} of solution into the water every day as described by the manufacturer. In this study *Spirulina platensis* (Cyanotech Corp., Hawaii, USA) was used as the spirulina additive.

Except for Group 4, all other groups used a basal ration containing 2% spirulina to investigate the difference between EM and 2% spirulina by comparing it to Group 2. The experimental design was presented (Table 4).

Previously, three lengths (horizontal I, horizontal II, and vertical) were measured in the literature to evaluate fish growth (Çelik et al., 2008; Erol et al., 2006; Hekimoğlu, 2001). The first measurement was taken from the anterior end to the caudal fin's peduncle (horizontal I), the second from the anterior end to the posterior end (horizontal II), and the third from the dorsal fin to the anal fin (vertical) by millimetric paper. Fish were also weighted by a calibrated scale. Measurements were taken on the starting of the study (T0), 7th (T1), 14th (T2), 21st (T3) and 28th (T4) days. Weight gain rate (WGR), length gain rate (LGR) and feed conservation rate (FCR)(Song et al., 2017), body index in terms of condition factor (CF)(Liu et al., 2019); specific growth rate (SGR, % day^{-1}) Ergün et al. (2009) were calculated as follows:

$$WGR\ (\%) = \frac{[\text{Final weight (g)} - \text{Initial Weight (g)}]}{\text{Initial Weight (g)}} \times 100$$

Vertical and Horizontal $_{I\text{-}II}$ Length Gain Rate (LGR) was calculated as described below:

$$LGR\ (\%) = \frac{[\text{Final Lenght (cm)} - \text{Initial Lenght (cm)}]}{\text{Initial Lenght (cm)}} \times 100$$

$$FCR = \frac{\text{Total Feed Weight}/10\ (\text{g})}{(\text{Final weight (g)} - \text{Initial Weight (g)})}$$

$$CF = \frac{\text{Final weight (g)}}{\text{Final Lenght (cm)}^3} \times 100$$

$$SGR\ (\%) = \frac{\ln \text{Final Weight (g)} - \ln \text{Initial Weight (g)}}{\text{Total Expremint (days)}} \times 100$$

The aquarium environment was critical for the successful growth of juvenile Discus. As a result, environmental quality indicators were closely monitored. The pH and conductivity levels were measured daily. To determine whether the variables were normally distributed, visual and analytical methods were used, and Levene's test was used to ensure variance equality. Grubb's Test was used to check the outliner of all length measurements and weights taken in T0. pH and conductivity concentrations were evaluated by using general linear models repeated measures procedure, one-way ANOVA test was used to check the mean WGR, Vertical and Horizontal $_{\text{I-II}}$ LGR, FCR, CF, SGR parameters and Tukey HSD post hoc test was used for the determination of group differences. The limit of statistical significance was assumed as $p < 0.05$. Statistical analyses were conducted with IBM® SPSS® (V. 21) and Blue Sky Statistics v10.3.2.

Results and Discussion

There was no outliner measurement in T0, and the study began with individuals that were all the homogeneous length and weight. The mean pH level in G1 was higher than in other treatment groups and conductivity was lower in Group 2 ($p<0.01$) (Table 5; Fig.1). In first and third week, mean conductivity values in G1 and G2 were higher than other groups ($p=0.002$) (Table 5). Mean pH level was higher in G_1 than other groups ($p<0.01$). Conductivity level was lower in Group 2 compared to other groups ($p<0.01$). Mean conductivity values were higher in G_1 and G_2 than other groups in first and third weeks ($p<0.002$) (Table 5).

Table 4. Experimental design

Group	Daily Treatment	Aim
Group 1 (Control)	Basal Ration + 2% *Spirulina* + Prevention Medicine	Routine breeding procedure as control
Group 2	Basal Ration + 2% *Spirulina* + Prevention Medicine + EM	Comparing if EM additive improve routine procedure
Group 3	Basal Ration + 2% *Spirulina* + EM	Can we use EM instead of Prevention Medicine
Group 4	Basal Ration+ 2% Prevention Medicine + EM	Can we use EM instead of Protein resource

Table 5. Effect of treatment and time on pH and conductivity (µscm^{-1})

	Weeks (Mean ± SD)				SEM	*P value*	
	First	Second	Third	Fourth		Group	Time
pH							
G_1	7.29±0.09^{a}	7.17±0.08^{a}	7.29±0.09^{a}	7.23±0.11^{a}	0.01		
G_2	7.03±0.05^{b}	7.03±0.08^{b}	7.03±0.05^{b}	7.03±0.08^{b}	0.01	0.01*	0.47
G_3	7.06±0.08^{b}	7.10±0.10^{ab}	7.06±0.08^{b}	7.09±0.11^{b}	0.02		
G_4	7.11±0.09^{b}	7.06±0.08^{ab}	7.04±0.08^{b}	7.06±0.08^{b}	0.01		
Conductivity							
G_1	386.29±6.07^{Aa}	361.86±13.63^{Ba}	381.14±10.53^{Aa}	361.86±13.63^{Ba}	2.94		
G_2	357.14±5.67^{Ab}	343.71±14.55^{ABb}	357.14±5.67^{Ab}	341.57±11.72^{Bb}	2.29	0.01*	0.002*
G_3	376.57±16.60^{a}	367.57±11.53^{a}	376.57±16.60^{a}	376.57±16.60^{a}	2.86		
G_4	382.86±13.18^{a}	375.43±11.96^{a}	382.86±13.18^{a}	372.57±11.67^{a}	2.39		

*SEM: Standard Error of Means; Tret.: Treatment Effect; Time: Time Effect; P: Probability; A,B: Means with different superscripts in the same row are different ($p<0.05$); a,b: Means with different superscripts in the same column are different ($p<0.05$). *: Statistically significant.

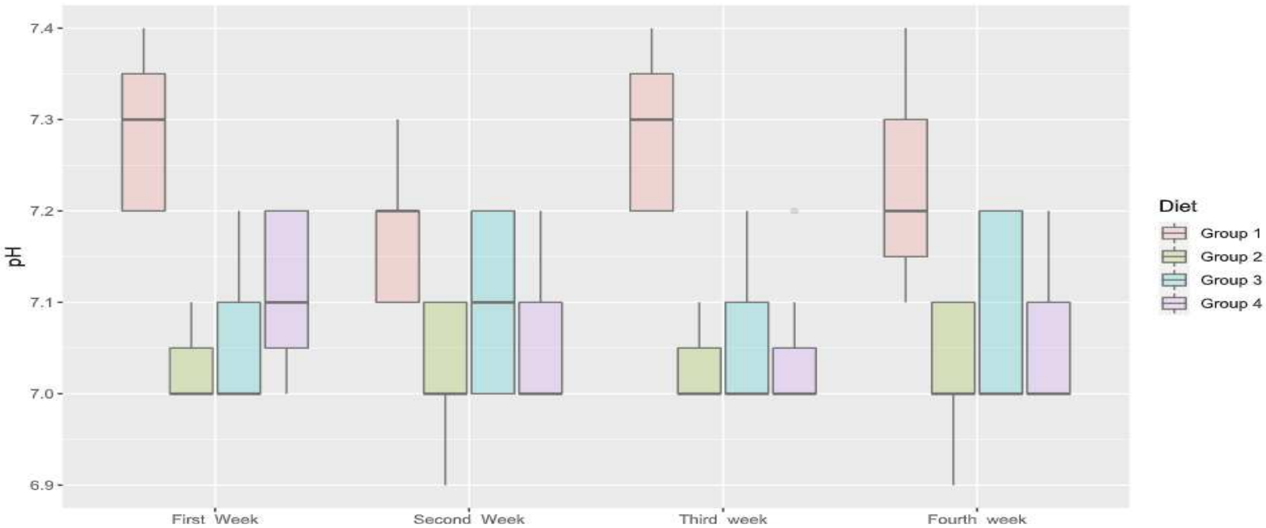

Fig. 1. Box-plots for weekly mean pH concantrations of the experimantal unit's.

Table 6. Weight, horizontal$_{\text{I-II}}$, vertical length gain, feed conservation rate, condition factor results.

Parameter	Group	Mean±SD	Median	SEM	(Min-Max)	pvalue
Weight Gain (g)	G1	5.63±0.34 ^{b}	5.75	0.11	(4.90-5.95)	0.001*
	G2	6.06±0.33 ^{a}	6.00	0.10	(5.50-6.60)	
	G3	6.37±0.37 ^{a}	6.3	0.12	(5.90-6.90)	
	G4	6.26±0.22 ^{a}	6.2	0.07	(6.00-6.60)	
Weight Gain Rate (%)	G1	6.17±0.23^{b}	6.25	0.08	(5.68-6.37)	0.001*
	G2	6.43±0.20 ^{a}	6.4	0.07	(6.09-6.74)	
	G3	6.61±0.21 ^{a}	6.58	0.07	(6.34-6.90)	
	G4	6.55±0.13 ^{a}	6.52	0.04	(6.40-6.74)	
Feed Conservation Rate (%)	G1	1.20±0.08^{a}	1.17	0.03	(1.13-1.38)	0.001*
	G2	1.12±0.06 ^{b}	1.12	0.02	(1.02-1.23)	
	G3	1.06±0.06 ^{b}	1.07	0.02	(0.98-1.14)	
	G4	1.08±0.04 ^{b}	1.09	0.01	(1.02-1.12)	
Condition Factor	G1	3.97±0.35	3.97	0.11	(3.43-4.50)	0.72
	G2	3.91±0.26	3.82	0.08	(3.61-4.32)	
	G3	3.83±0.22	3.77	0.07	(3.52-4.33)	
	G4	3.94±0.25	3.85	0.08	(3.70-4.31)	
Spesific growth rate (% day^{-1})	G1	4.84±0.22	4.89	0.07	(4.3-5.14)	0.07
	G2	4.98±0.19	5.02	0.06	(4.72-5.31)	
	G3	5.07±0.23	5.06	0.07	(4.78-5.47)	
	G4	5.02±0.14	4.97	0.04	(4.78-5.23)	
Horitanzal I Length Growth Rate	G1	2.37±0.12	2.39	0.04	(2.18-2.54)	0.55
	G2	2.45±0.09	2.48	0.03	(2.30-2.60)	
	G3	2.43±0.16	2.39	0.05	(2.25-2.72)	
	G4	2.39±0.12	2.42	0.04	(2.19-2.53)	
Horitanzal II Length Growth Rate	G1	1.90±0.11	1.92	0.03	(1.73-2.08)	0.46
	G2	1.96±0.07	1.96	0.02	(1.84-2.08)	
	G3	1.95±0.08	1.95	0.03	(1.81-2.04)	
	G4	1.92±0.12	1.94	0.04	(1.67-2.08)	
Vertical Length Growth Rate	G1	2.24±0.17 ^{b}	2.27	0.05	(1.87-2.42)	0.001*
	G2	2.43±0.1 ^{a}	2.42	0.03	(2.30-2.60)	
	G3	2.50±0.13 ^{a}	2.48	0.04	(2.36-2.72)	
	G4	2.46±0.11 ^{a}	2.42	0.03	(2.30-2.60)	

SEM: Standard Error of Means; P: Probability; a,b, ab: Means with different superscripts in the same column are significantly different according to Tukey HSD post hoc test; *: $p<0.05$, Statistically significant.

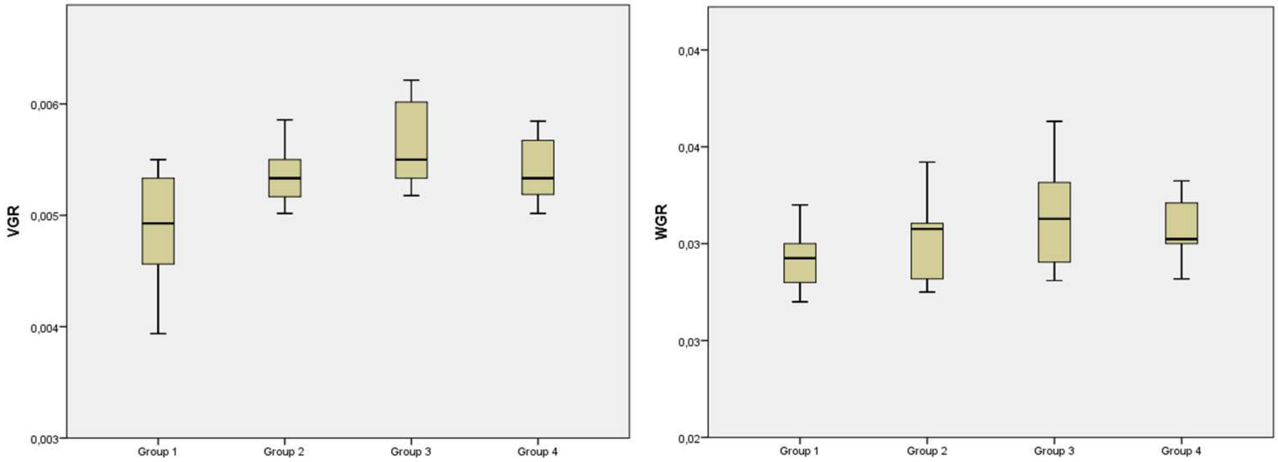

Fig. 2. Box-plots of the groups vertical gain rate (VGR) and weight gain rate (WGR).

All mean length and weight values were higher in G_4 on T1, in G_3 on 14th day, in G_3 and G_4 on 21th day, in G_2, G_3 and G_4 on 28th day than control group($p<0.05$). Horizontal I and II length means were higher in G_3 and G_4 than G_1 and G_2 ($p<0.001$) on the 14th day. G_3 had higher, G_1 had lower vertical measurements than other groups ($p<0.001$). WGR, VLGR, and $HLGR_{I-II}$ ratios were presented (Table 6 & Fig 2). VLGR and WGR mean values of the control group were significantly lower than other groups. FCR was 12% higher in control group comparing to G3. Experimental groups' FCR findings also supported WGR. Group 1 FCR was founded slightly higher comparing to the other groups ($p< 0.05$) (Table 6).

No deaths have been observed in any of the aquariums. A slimy structure was observed for 3-4 days in the tanks without plants in which EM was used and then the water became normal. It was observed that the fish consumed feed with more appetite in the EM groups. Although no significant changes were observed in colouration and pigmentation, it was observed that body mucosa secretion was more in EM supplemented groups.

EM was invented to be used in organic farming, but it is now finding wider applications in medicine, the environment, the livestock sector, forestry, and agriculture(Ahn et al., 2014; Higa& Parr, 1994). There is rare research about ornamental fish applications. In this research EM application effects on Discus fish was evaluated. In this study, fish space was as eight individual L^{-1} and this space was sufficient for objective determination of the growth parameters. Water temperature, nitrite and nitrate levels were observed to be optimum for discus breeding according to Çelik et al. (2008).

Water quality is the one of the most important confluents of stress factor of the fish, and it can play a key position in maintaining health in the aquarium. Discus is sensitive to diseases and environmental conditions such as pH, conductivity, nitrogen and ammonium levels (Çelik et al., 2008; Livengood et al., 2009). Water pH were determined between 7.02 - 7.28 in this study. Çelik et al. (2008) have recommended the pH level for Discus breeding to be between 5-6, but they also found that breeding could be performed between pH levels of 3.9 and 7.3. In this study, pH was more alkaline than these recommendations, but it is still within the reference values. In contrast, compared to control group, EM supplement groups had lower pH levels ($p<0.01$) and complementary to the recommended values that (Çelik et al., 2008) mentioned. Previously reported that effective microorganisms can produce lactic acid, acetic acid, and propionic acid through metabolism, which can lower the pH in water (Szymanski & Patterson, 2003) and in the colon and allow beneficial bacteria to predominate (Zhou et al., 2009). Similarly, pH level was decreased in EM groups compared to control group in this study. pH 6.6 was considered as a optimum Discus egg production pH (Swain et al., 2020). EM may have beneficial effects on growth of the eggs which may be considered for further studies.

Conductivity is another essential parameter for discus aquariums, especially for mating. This vital parameter was measured to be between 341.57 and 386.28 μscm^{-1}. Conductivity levels fluctuated by time effect in control and G2 groups. In the first and second-week, mean measurement values were higher than other weeks ($p<0.05$). Conductivity parameter was also affected by the treatment influence. Calcium, magnesium and total hardness influence conductivity (Hanekom et al., 2001). It is important for metal ions to be present in the environment for increasing conductivity in the aquariums. Supporting G2 with EM resulted in the decrease of the conductivity level compared to other groups ($p<0.05$). Szymanski & Patterson (2003) have mentioned that conductivity levels started to decrease after two-weeks due to the consumption of nutritive materials by fish. In G2, conductivity levels present an intermittent progress as a result of chemical manipulations like preventive medicine. In G3 and G4, conductivity measurements have stabilized with time. Çelik et al. (2008) indicated that breeding behavior could be seen under 300 μscm^{-1}. Conductivity can change as a result of various effects, and it should be monitored continuously, especially when using EM in mating aquariums. Water ion concentration was within normal limits when EM was used. In the future, specific ions can be measured and observed for their association with the EM supplementation.

WG, WGR decreased in control group, not horizantal I-II but vertical LGR and FCR were increased in control group. According to our findings we can clearly result that EM increased feed utilizion. G3 (Basal Ration + 2% Spirulina + EM) had the lowest FCR. According to our findings, this could be another important issue for future research. Survival rate, body composition, and color are substantial parameters in ornamental fish trade. Feed costs make up the highest proportion (~60%) of an intensive aquaculture production regimen (Ergün et al., 2009). FCR results showed us EM using have promising effects on decreasing the feed costs. Previously, it was concluded that beneficial microroganisim can decrease FCR in aquaculture (Zhou et al., 2009). The addition of EM bokashi (powder version) to crayfish diets had no effect on growth parameters, but it did increase survival rate (Özdoğan et al., 2022). In comparison to our results, the EM type may have an effect on showing the effects of it.

It was observed that epidermal mucus secretion was more in EM supplemented groups. Satoh et al. (2018) provided clear evidence that epidermal mucus provisioning functions as a form of parental nourishment and decreasing feed costs of Discus fish and increase survival. According to results, beneficial microorganism additives can increase mucus production, and this topic may be important for future research.

Previously, probiotic procurement methods have been explained by (Balcázar et al., 2006), and bathing have been showed as a way of probiotic addition. *Spirulina* additive is still essential for Discus breeding as a color and protein supplement; besides that, EM can decrease the use of medications or materials used for water quality, and this will make aquarium systems more environmentally friendly; therefore, the threat posed towards public health can be reduced. Probiotics inhibit pathogenic bacteria both in vitro and in vivo by several ways (Balcázar et al., 2006). As an example, lactic acid producing microorganisms also produce inhibitor substances such as acidollin, lactosidin, nicin and hydrogen peroxide, resulting in the decrease of harmful microorganisms in the environment (Alak & Atamanalp, 2012). Probiotics promote digestive characteristic of feeds, increase the absorption of vitamin and minerals, develop a barrier specifically for the intestinal epithelium and indigestible oligosaccharides, and some substances sustain the development of probiotic microflora (Sağdıç et al., 2004).

Since the study was carried out in aquariums without plant decor and in an environment with artificial lighting, it is not known whether it will give repeatable results for aquariums under sunlight and with plants. It would be better to take the study as a model only for the specified age group and developmental stage. Different environmental and physiological changes may be required in different age groups. Microbiological changes could not be evaluated in this study for financial reasons. On the other hand, adjusting the population of algae in water bodies, inhibiting the development of fish diseases as well as putrefaction of some aquatic plants during summer, boosting the immune system of aquatic animals, suppressing the harmful effects of oxidation through generating antioxidant substances and deactivate the occurring free radicals are attributed to beneficial organisms (Safwat & Matta, 2021). Further studies, monitoring the aquatic, fish mucosa and intestinal microflora changes will provide detailed information about the working mechanism of EM-1.

Conclusion

In conclusion, the use of EM-1 as a probiotic in Discus feed and spirulina in juvenile Discus feed, improved the FCR, WGR and VLGR compared to the control group. Medication and related to husbandry costs can be reduced by using EM, but more analyses need to be performed on survival.

EM use in planted aquariums and effects on the mating behavior should be tested before using in these conditions.

Reference

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