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APPLICATION OF DIETARY PROBIOTIC (VIBACT) LEVEL ON REPRODUCTIVE PERFORMANCE IN ICE BLUE CICHLID (MAYLANDIA GRESHAKEI)

[1]*P. Ambika, [2]J. Ronald and [3]A. Pushparaj

[1,2]Research Department of Zoology, St. Xavier's College, Palayamkottai, Tamil Nadu.

[3]Department of Zoology, TDMNS College, T. Kallikulam.

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***Corresponding Author**
P. Ambika
Research Department of Zoology, St. Xavier's College, Palayamkottai, Tamil Nadu.

ABSTRACT

In this study, we investigated the influence of probiotic (Vibact) supplementation on reproductive performance of developing ice blue cichlid (*Maylandia greshakei*) larvae. The probiotic feed V2 fed fish exhibited the maximum average fecundity per female (22.13±9.89), followed by V3 (19.7±8.36), V1 (18.14±9.19) and V4 (17.21 ± 9.2). A significant (P<0.05) decline in the average fecundity per female (14.23±6.19) was observed in fish fed the control feed (experimental group V0). The fish fed with the probiotic feeds V2 shows the higher fry survival (21.11±8.23), weight (0.76±0.04), length (41.31±1.10), fry weight (0.0026±0.003) and fry length (6.38±0.53) followed by V3 survival (17.82±7.65), weight (0.71±0.12), length (39.67±1.14), fry weight (0.0023±0.0002) and fry length (6.19±0.56), V1 survival (16.12±8.12), weight (0.74±0.11), length (39.56±1.83), fry weight (0.0024±0.0002) and fry length (6.11±0.51) and V4 survival (15.85±9.31), weight (0.68±0.10), length (39.37±1.11), fry weight (0.0022±0.0002) and fry length (6.14±0.63). All the parameters exhibited significant differences (P<0.05) within the probiotic diet fed fish. Likewise, the GSI of fish fed the probiotic feeds V2 has the higher range (9.67±0.54) followed by V3 (9.4±0.4), V1 (9.38±0.39) and V4 (8.81±0.30) were also significantly higher (P<0.05) than fish fed the control diet V0 (8.11±0.42). All the probiotic-fed fish exhibited significantly lower. The probiotic feed V2 fed fish exhibited the maximum average hatching and survival rate per female (97.41 and 97.67), followed by V3 (92.80 and 91.90), V1 (89.13 and 88.50) and V4 (85.44 and 84.33) throughout the experimental period. A significant (P<0.05) decline in the average hatching and survival rate per female (83.14 and 82.14) was observed in fish fed the control feed (V0).

KEYWORDS: Maylandia greshakei, spawning, fecundity, probiotics.

INTRODUCTION

Ornamental fish keeping is one of the most popular hobbies in the world today. The commercial production of ornamental tropical fish is gaining momentum as a global component of international trade fisheries and aquaculture development. The last four decades have witnessed considerable growth and diversification in the international trade of ornamental fish. Now-a-days, "Probiotics" is defined more precisely as "mono or mixed cultures of live microorganisms which, when applied to animal, beneficially affect the host by improving the properties of the indigenous micro flora". The term "Probiotic" inevitably refers to Gram-positive bacteria associated with the genus *Lactobacillus.* However, nowadays, there has been a renewal of interest in the use of probiotics. In general terms, a group of requirements have been identified as important properties for *Lactobacilli* to be effective probiotic organism. These include the ability to adhere to cells, exclude or reduce pathogenic adherence, persist and multiply, produce acid, resist vaginal microflora, be safe and therefore noninvasive, noncarcinogenic and nonpathogenic and, co-aggregate and form a normal.

In recent years probiotics have been increasingly used in the biological control to prevent diseases in aquaculture. Currently, commercial products are available in liquid, Capsule, tablet or powder presentations and various technologies have been developed for improvement. The probiotic microorganisms have an antimicrobial effect and it has the capacity to alter the intestinal microbiota by producing antibacterial substances. These antibacterial substances overcome with pathogens to prevent their adhesion to the intestine, to restrict the nutrients necessary for pathogen survival by producing an antitoxin effect. Probiotic bacteria used as dietary additives seem to offer an attractive choice including overall health benefits to the host organism.[1]

In this context the present investigation was designed to evaluate the efficacy of commercial probiotic (Vibact) on reproductive performance of female freshwater ornamental ice blue cichlid, *Maylandia greshakei.*

MATERIALS AND METHODS

Experimental Setup

The experimental fish *M. greshakei* (Ice blue cichlid) is an omnivorous freshwater ornamental fish was chosen for the present study. It belongs to the genus *Maylandia*, known under the common name Ice blue cichlid. It inhabits in fresh water and successfully establishes in a variety of environmental conditions. It can grow to a maximum length of one meter.

The experimental fish ice blue cichlid (*M. greshakei*) of about 4 months old juveniles were purchased from a commercial fish farm (Saravanan fish farm) in Sawyerpuram at Thoothukudi District. The collected fishes were transported to laboratory in a polythene bag with aerated water. In the present study, the experimental fish having a length 3 - 4 cm approximately were randomly selected for the study. The chosen fishes were transferred to a cement water tank and acclimatized to the laboratory conditions for a period of 15 days. After that the experimental animal were transferred to the plastic troughs and water was changed once in two days. The experimental diets were feed thrice a day (9am, 1pm and 5pm). Water quality parameters like pH, dissolved oxygen, temperature, total alkalinity and ammonia were measured periodically following standard methods.[2]

Probiotics

For the present study, a commercial probiotic vibact has been selected. It contains *Lactobacillus* as an active ingredient. It helps to suppressing or killing the harmful bacterial growth in the medium. The vibact tablet contains mixture of bacterial culture including *Streptococcus faecalis* (30 million), *Clostridium butyricum* (2 million), *Bacillus mesentericus* (1 million), *Lactic acid bacillus* (50 million).

Probiotic Diet Preparation

The selected probiotic vibact was supplemented to the basal diet of 100gm at the levels of 0.2, 0.4, 0.6 and 0.8g. The basal diet was prepared by using the following ingredients like wheat flour, soybean, groundnut oil cake, fish meal and corn flour. The experimental diets were prepared by thoroughly mixing the dry ingredients with water, steam sterilized and after cooling 0.2, 0.4 and 0.6 and 0.8 gm of probiotic vibact were added to the basal diet and soft dough was prepared. This was then passed through a mincer and the obtained pellets were dried properly. To avoid fungal infection, these pellets were packed in an air tight polythene

bags. The proximate compositions like moisture, protein, lipid and ash of all probiotic feeds and control feed were determined using standard procedures of AOAC.[3]

Experimental design

About 60 days feeding trial was conducted in laboratory in circular plastic tubs. Before started to feed the experimental diet the initial weight of all the fishes were noted. The experimental fishes were divided into four groups and the groups were fed with different concentrations of 0.2 (V1), 0.4 (V2), 0.6 (V3) and 0.8 gm (V4) of probiotic (Vibact tablet) supplemented feeds during the experimental period. The proximate composition of control and different experimental diets was given in Table 1. The experimental feeds were given to the fishes twice a day and the control group was also maintained. The experimental fish were fed with feed at 5% of their body weight and also fed with two split doses throughout the experimental period. Excess feed should be avoided and also the unutilized feed and fecal matter were collected before every morning and water was changed once in every two days to maintain the water quality parameters.

Table 1. Proximate composition of control and different experimental diets.

Proximate composition	V0	V1	V2	V3	V4
Moisture	34.23	34.72	34.53	34.15	35.26
Protein	4.21	5.26	5.49	5.23	4.93
Lipid	10.84	10.47	10.54	10.87	10.57
Ash	8.93	8.77	9.08	9.08	8.44

Values are represented as mean.

5.2.5 Parameters studied

Reproductive parameters such as Relative Fecundity (RF), Gonado Somatic Index (GSI), Fry Survival, dead fry, deformed fry, weight and length of fry and weight and length of adult by using the following formula. All data obtained from experiments were analyzed by One-way analysis of variance (ANOVA) to compare the significance between control and experimental diets. Significance was considered at 5% ($P<0.05$) level.

$$\text{Relative Fecundity} = \frac{\text{Total fry production throughout experimental period}}{\text{Mean weight of female in Gram}}$$

$$\text{Gonado Somatic Index} = \frac{\text{Ovary weight}}{\text{Body weight}} \text{ X } 100$$

$$\text{Fry Survival} = \frac{\text{Total live fry after time}}{\text{Total fry production}} \text{X}100$$

$$\text{Hatching Rate} = \frac{\text{Total Number of fry}}{\text{Experimental period}} \text{ X } 100$$

$$\text{Survival Rate} = \frac{\text{Total Number of fishes introduced}}{\text{Number of fish survival}} \text{ X } 100$$

RESULTS

Assessment of reproductive performance

The table 2 shows that the results of reproductive performance of different experimental groups of *M. greshakei.* The average weight and length of spawning females were highest in fish of experimental group V2 and differed significantly (P<0.05) from the spawning females of the other experimental groups. With an increase in the concentration of probiotic supplementation in feed, the average fecundity and fry survival in *M. greshakei* increased. The probiotic feed V2 fed fish exhibited the maximum average fecundity per female (22.13±9.89), followed by V3 (19.7±8.36), V1 (18.14±9.19) and V4 (17.21 ± 9.2). A significant (P<0.05) decline in the average fecundity per female (14.23±6.19) was observed in fish fed the control feed (experimental group V0). The fish fed with the probiotic feeds V2 shows the higher fry survival (21.11±8.23), weight (0.76±0.04), length (41.31±1.10), fry weight (0.0026±0.003) and fry length (6.38±0.53) followed by V3 survival (17.82±7.65), weight (0.71±0.12), length (39.67±1.14), fry weight (0.0023±0.0002) and fry length (6.19±0.56), V1 survival (16.12±8.12), weight (0.74±0.11), length (39.56±1.83), fry weight (0.0024±0.0002) and fry length (6.11±0.51) and V4 survival (15.85±9.31), weight (0.68±0.10), length (39.37±1.11), fry weight (0.0022±0.0002) and fry length (6.14±0.63). All the parameters exhibited significant differences (P<0.05) within the probiotic diet fed fish. Likewise, the GSI of fish fed the probiotic feeds V2 has the higher range (9.67±0.54) followed by V3 (9.4±0.4), V1 (9.38±0.39) and V4 (8.81±0.30) were also significantly higher (P<0.05) than fish fed the control diet V0 (8.11±0.42). All the probiotic-fed fish exhibited significantly lower.

Table 2: Reproductive performance of different experimental groups of *M. greshakei.*

Experiment groups	Experimental Groups				
	V0	V1	V2	V3	V4
Fecundity	14.23±6.19c	18.14±9.19ab	22.13±9.89a	19.7±8.36ab	17.21±9.2b
Fry survival	11.71±4.71c	16.12±8.12b	21.11±8.23a	17.82±7.65ab	15.85±9.31b
Dead fry	2.46±2.18b	1.83±1.76a	1.03±1.27a	1.64±1.32a	1.15±1.31a
Deformed fry	0.30±0.74b	0.22±0.52ab	0.3±0.32a	0.14±0.34ab	0.20±0.53ab
Weight (g)	0.69±0.06ab	0.74±0.11c	0.76±0.04a	0.71±0.12ab	0.68±0.10b
Length (mm)_	38.16±1.03a	39.56±1.83b	41.31±1.10a	39.67±1.14a	39.37±1.11a
Fry weight (g)	0.0018±0.0001b	0.0024±0.0002a	0.0026±0.003a	0.0023±0.0002a	0.0022±0.0002a
Fry length (mm)	5.32±0.63d	6.11±0.51a	6.38±0.53c	6.19±0.56ab	6.14±0.63bc
GSI (%)	8.11±0.42b	9.38±0.39a	9.67±0.54a	9.4±0.4a	8.81±0.30ab

Average per female.

Mean - SD values with different letters in each row are significantly (P<0.05) different.

The table 3 deals about the spawning details of the different experimental groups of the experimental fish. The probiotic feed V2 fed fish exhibited the maximum average fecundity per female (22.13±9.89), followed by V3 (19.22±8.36), V1 (18.70±9.19) and V4 (17.44±9.2) throughout the experimental period of 60 days. A significant (P<0.05) decline in the average fecundity per female (14.23±6.19) was observed in fish fed the control feed (experimental group V0).

Table 3: Spawning details of *M. greshakei* during the study period.

Pair	Study Period (Days)	Total fecundity	Number of eggs/female		
			Max	Min	Average
V0	60	07	16	08	11.71
V1	60	10	20	17	18.70
V2	60	12	24	17	20.08
V3	60	09	20	16	19.22
V4	60	09	19	13	17.44

Values are represented as mean + SE.

The table 4 and 5 shows that the average hatching and survival rate of the experimental fish. The probiotic feed V2 fed fish exhibited the maximum average hatching and survival rate per female (97.41 and 97.67), followed by V3 (92.80 and 91.90), V1 (89.13 and 88.50) and V4 (85.44 and 84.33) throughout the experimental period. A significant (P<0.05) decline in the average hatching and survival rate per female (83.14 and 82.14) was observed in fish fed the control feed (V0).

Table 4: Average hatching rate of *M. greshakei* during the experimental period.

Pair	Temp (°C)	No. of Spawn	Hatching Rate												Average
			1	2	3	4	5	6	7	8	9	10	11	12	
V0	29±2	07	83	84	82	80	84	87	82	-	-	-	-	-	83.14
V1	29±2	08	88	89	87	91	89	90	91	88	-	-	-	-	89.13
V2	29±2	12	97	98	98	97	100	97	98	96	98	96	97	97	97.41
V3	29±2	10	90	90	95	93	93	94	94	91	94	93	-	-	92.80
V4	29±2	09	86	84	88	85	83	86	87	86	84	-	-	-	85.44

Values are represented as mean + SE.

Table 5: Average Survival rate of *M. greshakei* during the experimental period.

Pair	Temp (°C)	No. of Spawn	Survival Rate												Average
			1	2	3	4	5	6	7	8	9	10	11	12	
V0	29±2	07	83	82	82	84	80	81	83	-	-	-	-	-	82.14
V1	29±2	08	88	90	88	89	88	89	86	90	-	-	-	-	88.50
V2	29±2	12	97	96	97	98	98	99	98	99	98	97	97	98	97.67
V3	29±2	10	91	90	93	92	94	92	94	90	93	90	-	-	91.90
V4	29±2	09	84	85	84	84	86	86	85	81	84	-	-	-	84.33

Values are represented as mean + SE.

DISCUSSION

The findings of this study revealed that incorporation of probiotics in feed favorably influenced the reproductive performance of the experimental fish *M. greshakei* in terms of high fecundity, high fry survival, reduction in fry mortality and deformity, high gonado somatic index and higher average weight and length of fries. The probiotic bacteria has the capacity to establish in the gut to enhance larval nutrition and brood stock by producing essential nutrients like protein and essential fatty acids and some important enzymes namely amylase, protease and lipase.[4] Probiotic bacteria in the fish intestine enhances host enzymes secretion, which increases the digestive efficiency of the complex protein and lipids included in the diet, thus increasing the rate at which they can be assimilated by the host animal.

Hernandez *et al.*,[5] analyzed the effects of the commercial probiotic, *Lactobacillus casei* on the growth and protein content of skin mucus and stress resistance of juveniles of the Porthole live bearer *Poecilia gracilis* (Poecilidae). Dharmaraj and Dhevendran[6] evaluated the efficacy of *Streptomyces* as a probiotic feed for the growth of ornamental fish, *Xiphophorus helleri.* Effect of probiotic immunogen on reproductive performance in female platy (*Xiphophorus maculatus*) was carried by Abasali and Mohamad.[7] A study was conducted to examine the effects of the probiotic *Lactobacillus rhamnosus*, as a feed additive, on zebrafish (*Danio rerio*) fecundity.[8] Mansa and Allah[9] studied the effect of dietary vitamin C on reproductive performance of a fresh water ornamental species the platy (*Xiphophorus maculatus*). Nekoubin *et al.*,[10] studied the effect of symbiotic (*Biomin imbo*) on fecundity and reproduction factors of zebra fish (*Danio rerio*). Effect of diet (fatty acid and protein) content during spawning season on fertility, eggs and larvae quality of common porgy was studied by Abrenouch *et al.*[11]

The present investigation showed highest fry survival in the probiotic supplemented fishes and maximum values were recorded in 0.4gm of probiotic incorporation during the

experimental period. These findings were in agreement with the findings of Abasali and Mohamad[7], who noticed a highest value of fry survival in platy, *Xiphophorus maculates* and also agreement with the findings of Chitra and Krishnaveni[12], who noticed a highest value of fry survival in black molly, *Poecilia spenops.*

The importance of vibact probiotic is to suppressing or killing the harmful bacterial growth in the experimental medium. It helps to influence on reproductive performance was possibly due to alternation of the fish intestinal microflora and improving the beneficial mixed bacteria culture by probiotic ingredients, particularly *Streptococcus faecalis, Clostridium butyricum, Bacillus mesentericus* and *Lactic acid bacillus*. Higher survival rate and lower deformed fry could be linked to the intestine probiotic bacterial population which produces B complex vitamins. Care should be taken when the probiotic levels administered to fish and to avoid overdosing and under dosing with resultant lower efficacy and unnecessary costs.[12] From this study, we concluded that a probiotic concentration of 0.4gm (V2) was sufficient for enhanced reproductive performance, and that the use of a higher concentration of probiotic cells did not always yield significantly better results. However, the use of a higher concentration of probiotic cells did not always yield significantly better results. The results showed that dietary probiotic supplementation improved the reproductive performance indices of the ice blue cichlid *M. greshakei*.

CONCLUSION

Our study was the first of its kind where the elects of an isolated probiont vibact on the reproductive performance of the selected experimental fish ice blue cichlid, *M. greshakei* were evaluated. Moreover, further studies are needed to determine the mechanisms responsible for the improvement of reproductive performance of the fish. There is an ever the application for probiotics in aquaculture will be beneficial especially for improving reproductive efficacy of the ornamental fishes to increasing commercial interest in the ornamental fish trade in all over the world. The field of probiotics intended for aquaculture organisms is now gaining much importance. The results of this study would release new avenues for research in other commercially important freshwater ornamental cichlid fish species in the days to come.

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