

Growth Promotion Effect of Triphala Bio-Fortification on *Oreochromis mossambicus*

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Abstract: The present study was conducted to evaluate the dose dependent bio fortification efficacy of methanolic extract of Triphala on *Oreochromis mossambicus* as reflected by growth parameters. (TD1=0.4%, TD2=0.8% and TD3=1.2% Triphala) The specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency rate and protein efficiency ratio, were assessed and documented in each terminations. Significant increase in SGR, optimum FCR, FER and PER were observed in Triphala supplementation group compared to control. FCR presented an inverse relation with SGR. Significant increase in percentage of survival observed in Triphala fortified group after *Aeromonas hydrophila* infection compared to control. Hence present study confirmed that Triphala fish feed preparations can act as growth promoter and immune booster

Keywords: Tilapia, *Oreochromis mossambicus*, *Aeromonas hydrophila*, Growth. Immunostimulants

1. Introduction

Exponential population growth rate coupled with demand for food fishes in the domestic and international market had stirred tremendous interest among aqua farmers, entrepreneurs and industrialist to experiment with new sustainable culture methods. The development of new species-specific fish feed formulations supports the intensive aquaculture industry. Disease management is a key problem and the same through antibiotics and chemicals lead to bacterial resistance and accumulation of unacceptable residues in aquaculture products and environment. (Sameeda et al. 2011, Jayasree et al., 2014, Jasmine, et al., 2016, Salini, et al., 2015).

Hence we have to look for alternative strategies. It has been proved beyond doubt that medicinal herb incorporated aqua feeds provide basic nutritional requirements to improve growth, feed utilization and also supports general health and stress resistance (Jayasree et al., 2013., 2016).

Tilapia proves a good candidate for commercial aquaculture because of its hardy nature and excellent efficiency to adapt to wide array of environmental changes and also of its readiness to accept on formulated feeds. Tilapia is highly nutritious and forms a part of the balanced diet, high in protein (16–25%) and low in fat (0.5–3.0%).

Triphala, is a herbal formula prepared by mixing the dried powder of *Embllica officinalis*, *Terminalia chebula*, and *Terminalia bellerica* (1:1:1 proportion). It purifies blood, stimulates bile secretion and detoxifies the liver. The efficacy of medicinal plants stick on precise dose hence in the present study dose dependent effect of Triphala, was attempted to evaluate its effect on growth and survival of *Oreochromis mossambicus*.

2. Materials and Methods

2.1 Experimental set up

The fruits of medicinal plants, *Embllica officinalis*, *Terminalia bellerica* and *Terminalia chebula* were collected and identified in Pankajakasturi Ayurvedic Research Centre, Thiruvananthapuram. *Oreochromis mossambicus* juveniles, 6.50±0.50 cm in length and 6.50±0.50 gm weight collected from Agency for Development of Aquaculture in Kerala at Varkkala. The fishes were quarantined and stocked at 20 fish / 1000 L water, and maintained at normal laboratory conditions for two weeks for acclimatization. Subsequently they were randomized in to four groups, one group served as control and was supplemented with control feed and other 3 groups were fed with medicated feed (TD1=0.4% Triphala, TD2=0.8% and TD3=1.2% Triphala incorporated diet) for two months. Both control and experimental fish were fed @ 2% of their body weight twice daily. During the experimental period the water quality variables: temperature (28±1°C), pH (7.2±0.2), and dissolved Oxygen (7.5 mg/l) were recorded. 50% water, fecal materials and unused feed were removed daily. The experimental regime continued, in triplicate and two terminations were made on 30th & 60th day and the growth parameters and survival % were assessed and documented.

2.2 Methanolic extract preparation

The methanolic extract preparation was done as outlined by Singh, (2008). Fresh fruits of *Embllica officinalis*, *Terminalia chebula*, and *Terminalia bellerica* were washed in sterilized water, shade dried, de seeded and grinded separately. Fruit powder was successively extracted with 85% absolute methanol and then subjected to vacuum filtration. The filtrate is dried and the residue obtained after evaporation was carefully collected, weighed and transferred into air tight containers and stored at -20°C till use.

2.3 Feed Preparation

The control diet was prepared by mixing fish meal 40gm, rice brawn 25gm, ground nut oil cake 10 gm, soya 10 gm, tapioca powder 5 gm, wheat flour 5 gm, with 100 ml sterilized water and cooked in pressure cooker for 5 minutes. Cooked feed is allowed to cool and fish oil 2 ml, vegetable oil 2 ml, and one vitamin & mineral mix tablet was added. The cooked feed is pelletized, sun dried and stored in glass jars. To prepare experimental diet methanolic extract of Triphala (0.4%, 0.8% 1, 2 %) was added to the control diet before pelletizing.

2.4 Challenge study

Subsequent to feeding regime both control and experimental fishes were challenged intra peritoneally with *Aeromonas hydrophila* (10^8 cfu/ml) and survival data were documented in pre and challenged control and experimental fishes. Specific Growth rate, Feed Conversion Ratio (FCR), Feed efficiency rate (FER) and Protein Efficiency Ratio (PER) were calculated using the equations below.

- 1) Specific growth rate (SGR, %/day) = $[(\ln W_t) - \ln (W_0) / T] \times 100$ (Ln- natural logarithm; W₀- initial weight; W_t- final weight; and T-time in day).

- 2) Feed conversion ratio (FCR) = Total dry feed consumed (gm) / Total wet weight gained in gm.
- 3) Feed efficiency rate (FER) = (Live weight gained (g) / dry feed given (g)) X 100
- 4) Protein efficiency ratio (PER) = Wet weight gained (g) / amount of protein consumed (g).

2.5 Statistical Analysis

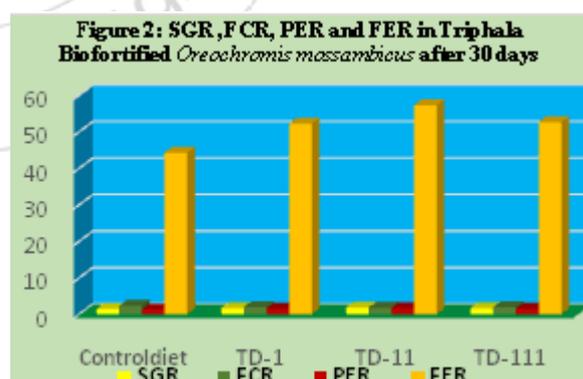
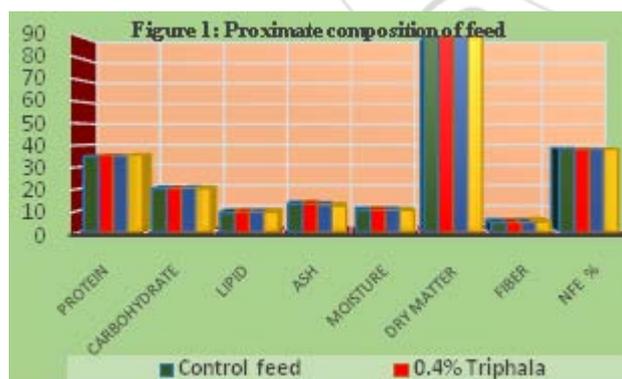
The statistical analysis was carried out using the software SPSS 17.0 package. For determining the significant difference between different treatments in blood and serum parameters one-way ANOVA followed by Duncan's test was done. Significance level (P value) was set at 0.05 in all tests. Values in the same row with different lower case letters vary significantly ($p < 0.05$) between treatment groups. Each value represents the mean \pm SE of six separate data.

3. Result and Discussion

The proximate biochemical composition of the diets were done to ensure proper proportion of nutrients and the results were given in Table 1.

Table 1: Proximate composition of feed

In 100 gm feed gm %	Control feed	Triphala TD-I	Triphala TD-II	Triphala TD-III
Protein	35.41 \pm 0.08 ^a	35.39 \pm 0.10 ^a	35.20 \pm 0.04 ^a	35.22 \pm 0.32 ^a
Carbohydrate	20.12 \pm 0.01 ^a	20.01 \pm 0.03 ^a	20.21 \pm 0.01 ^a	20.08 \pm 0.12 ^a
Lipid	10.18 \pm 0.18 ^a	10.18 \pm 0.24 ^a	10.15 \pm 0.17 ^a	10.13 \pm 0.12 ^a
Ash	12.84 \pm 0.22 ^a	12.98 \pm 0.18 ^a	12.55 \pm 0.12 ^a	12.64 \pm 0.17 ^a
Moisture	10.05 \pm 0.21 ^a	10.16 \pm 0.16 ^a	10.14 \pm 0.09 ^a	10.06 \pm 0.06 ^a
Dry matter	89.95 \pm 0.52 ^a	89.84 \pm 0.26 ^a	89.86 \pm 0.35 ^a	89.94 \pm 0.23 ^a
Fiber	4.39 \pm 0.09 ^a	4.38 \pm 0.04 ^a	4.58 \pm 0.06 ^a	4.53 \pm 0.07 ^a
NFE %	37.18 \pm 0.11 ^a	37.07 \pm 0.12 ^a	37.52 \pm 0.08 ^a	37.68 \pm 0.15 ^a
GE	448.5 \pm 0.01 ^a	448.1 \pm 0.12 ^a	448.5 \pm 0.01 ^a	448.22 \pm 0.11 ^a



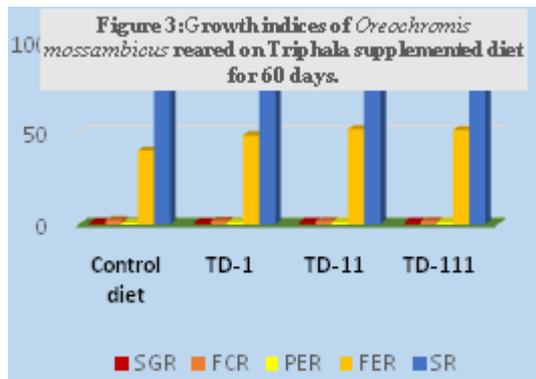


Table 2: Growth indices of Triphala Bio fortified *Oreochromis mossambicus*(30days)

Growth Indices	Control diet	Experimental Diet- Triphala		
		TD-I	TD-II	TD-III
Initial wt.(g)	6.50±0.30 ^a	6.48±0.36 ^a	6.52±0.39 ^a	6.61±0.38 ^a
Final wt. gm)	9.95±0.10 ^a	10.55±0.36 ^a	10.98±0.32 ^b	10.71±0.16 ^a
Wt. gain	3.45±0.01 ^a	4.07±0.08 ^b	4.46±0.02 ^c	4.10±0.01 ^b
Wt gain (%)	53.07±0.02 ^a	62.80±0.02 ^b	68.4±0.01 ^c	62.02±0.02 ^b
SGR	1.42±0.02 ^a	1.62±0.01 ^b	1.74±0.01 ^c	1.63±0.01 ^b
FCR	2.26±0.04 ^c	1.91±0.01 ^b	1.75±0.01 ^a	1.90±0.01 ^b
FER	44.23±0.01 ^a	52.17±0.03 ^b	57.17±0.03 ^c	52.56±0.02 ^b
PER	1.26±0.01 ^a	1.49±0.01 ^b	1.63±0.01 ^c	1.50±0.02 ^b

Highly reflective growth increment and survival was observed in *Oreochromis mossambicus* bio-potentiated with 0.8% Triphala incorporated feed and had significantly higher, SGR, optimum FCR, good FER, and PER than other supplementation group and the control (Table2). The improvisation can be attributed to the effect of active principles in the medicinal herbs incorporated in the diet manifested a synergistic effect. The active principles may enhanced feed utilization by the assimilation of most nutrients from diet and had to convert them into flesh. Present results are in agreement with Ahilan (2010) who reports that the inclusion of *Phyllanthus niruri* and *Aloe vera* as herbal feed additives had enhanced the growth performance of goldfish, *Carassius auratus* and disease resistance against *A. hydrophila* infections. Abutbul *et. al.* (2004) found that Tilapia fed with a diet containing ethyl acetate extract of *Rosmarinus officinalis* leaf powder had presented improved growth parameters. Asadi *et. al.*, (1996) found that *Phyllanthus niruri* and *Aloe vera* when supplemented to *L. rohita*, the *Phyllanthus niruri* fed group exhibited higher specific growth rate, because of improved feed utilization and high rate of protein synthesis. Glencross, *et. al.*, (2007) observed Yellow lupin at 12.5% included in fish diets of rainbow trout enhanced FCR. They anticipated that phytochemicals in these herbs might have promoted cellular lipid and fatty acid utilization and protein accumulation resulting in good growth performance.

Table 3: Growth indices of *Oreochromis mossambicus* reared on Triphala supplemented diet (60 days)

Growth Indices	Control diet	Experimental Diet- Triphala		
		60 days treatment		
		TD-I	TD-II	TD-III
Initial wt. (gm)	6.50±0.30 ^a	6.48±0.36 ^a	6.52±0.39 ^a	6.61±0.38 ^a
Final wt. (gm)	13.52±0.30 ^a	14.95±0.36 ^a	15.62±0.32 ^b	15.59±0.16 ^a
Average wt. gain	7.02±0.041 ^a	8.47±0.041 ^b	9.1±0.041 ^c	8.98±0.041 ^{bc}
Wt. gain (%)	108±0.12 ^a	131±0.06 ^b	139±0.02 ^c	138±0.01 ^c
SGR	1.22±0.041 ^a	1.39±0.027 ^b	1.46±0.23 ^c	1.44±0.019 ^{bc}
FCR	2.48±0.066 ^b	2.05±0.057 ^b	1.91±0.017 ^a	1.93±0.082 ^a
FER	40.34±0.07 ^a	48.7±0.094 ^b	52±0.224 ^d	51.6±0.056 ^{cd}
PER	1.15±0.02 ^a	1.39±0.020 ^b	1.49±0.022 ^d	1.47±0.020 ^c
SR	96.6±1.02 ^a	100±0.00 ^b	100.0±0.00 ^b	96.6±1.04 ^a

Table 4: Percentage of survival in Triphala bio fortified *Oreochromis mossambicus* subsequent to challenge with *Aydropthila*.

Test Diets	Survival Percentage	
	Pre challenge	Challenged
Control	96.6±1.02 ^a	53.3 ±1.15 ^a
TD-I	100.0±0.00 ^b	86.6 ±5.65 ^c
TD-II	100.0±0.00 ^b	86.6±5.65 ^c
TD-III	96.6±1.04 ^a	72.3±2.35 ^b

To summarize present study it can be conclusively stated that phytonutrients present in Triphala, had growth promoting effect with a preliminary suggestion for disease resistance against *Aeromonas hydrophila* in *Oreochromis mossambicus*. The secondary metabolites in Triphala, with strong credentials can be used in aquaculture to bio potentiate the culture species, *O. mossambicus* leading to sustainable, organic aquaculture strategy. Moreover, this study validates the optimum level of tested herbal extracts included in fish diet. (Triphala, 8gm/1kg feed). Current study reinforces the application of phytotherapy as a powerful tool for disease management in aquaculture, to reduce anthropogenic contamination within the aquatic environments and lay foundation for sustainable aquaculture.

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