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FUNCTIONAL FEED EFFICACY OF *SARACA INDICA* SUPPLEMENTED DIET ON *OREOCHROMIS MOSSAMBICUS*

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Abstract: Due to the intensification of rearing methods and systems, diseases and pathogens have been an integral part and formidable obstacle to the aquaculture industry worldwide. Although aquaculture production is growing rapidly, disease prevention and treatment practices are far from standardized or regulated .Habitual use of antibacterial can lead to problem with bacterial resistance and unacceptable residues in aquaculture products and environment. There has been an increasing incidence of multiple resistances in pathogenic microorganisms in recent years.Hence there is a need to look for eco-friendly disease preventive measures to promote sustainable aquaculture. Herbal supplements used in aqua feeds are harmless because they are natural product and therefore pose no threat to fish health and aquatic environment. Functional feeds extend beyond the satisfying basic nutritional requirements of the cultured organism to improve growth and feed utilization and also the support of general health and stress resistance of the animals. Present investigation was carried out to assess the alterations, if any in the microbial diversity and changes in biochemical turnover at certain tissues of *Oreochromis mossambicus* supplemented with *Saraca indica*. Tilapia juveniles, 7.50±0.50 cm in length and 6.50±0.50 gm weight were collected, quarantined and stocked at 20 fish / 1000 L water, and maintained at normal laboratory conditions. One group served as control and was given the basal feed&, second group was fed with medicated feed (0.8% *Saraca indica* included diet) prepared in the laboratory. Both groups were fed @ 2% of their body weight twice daily. The experiment continued, in triplicate for two months. On termination (30^{th}&60^{th}day) the tissues were processed as per standard protocols. The microbial species diversity at gut, gill, and skin surfaces, were assessed and documented. Results indicate a significant enhancement in the pro biotic microbial load in these sites and a marked decrease in the opportunistic microbial strains with respect to the control. Regarding nitrogen excretion it showed a better nitrogen excretion efficiency. The data has been statistically evaluated, discussed and reported. Present study can be concluded by stating that methanolic extract of *Saraca indica* is quiet effective as an antimicrobial agent had improved nitrogen excretion efficacy, and is non toxic. The aquatic ecosystem can be effectively saved from the harmful effect of antibiotics used in the culture system.

Key words: Functional feed, Microbial diversity, Nitrogen excretion and antibiotics.

INTRODUCTION

Aquaculture is one of the fastest growing industries especially in developing countries like India, however **i**ts progress is hampered by the outbreak of various diseases. Although the application of antibiotics and chemicals can be effective in controlling pathogens in aquatic animal farms, the residues of these compounds in meat may be a threat to the health of consumers (Biswas *et al*., 2010). Antibiotic therapy is undesirable, as there is the potential for enhanced microbial resistance and the accumulation of residues in the tissues of the fish (Siwicki, 1989). Recently, several efforts have been made to replace chemical drugs by herbal medicine in aquaculture industry in many countries (Dugenci *et al*., 2003; Citarasu *et al*., 2006; Obaroh and Achionye-Nzeh, 2011). In fact, most research has focused on the role of plant extracts in stimulating the immune system in fish comparing with bacterial, parasitic and fungal agents (Sivaram *et al*., 2004; Rao and Chakrabarti, 2005; Citarasu *et al*., 2006; Rao *et al*., 2006; Divyagnaneswari *et al*., 2007; Sarkar *et al*., 2011).

In recent yars, the use of medicinal plants as an effective alternative to antibiotics has gained importance, especially to combat disease problems in fishes (Sudhakaran *et al*., 2006).

Plant derived phyto medicines have great promise in the treatment of infectious diseases. Herbal products promise a cheaper source for therapeutics, greater accuracy than chemotherapeutic agents and a viable solution for all problems which fish culture faces today.

Tilapia is an important model for studying of fish physiology, particularly because of its broad tolerance to an array of environments. Currently Tilapia are the second most farmed fish in the world with an annual production exceeding 2.8 million tons in 2010.They exhibit a versatile adaptability to different environmental conditions to match the vast array of their ecological habitats. *Saraca indica* is highly regarded as a universal panacea in the Ayurvedic medicine. Flower contains Oleic, linoleic, palmitic and stearic acidssitosterol, quercetin, kaempferol, quercetin apigenin- 7-o-p-D-glucoside, Pelargonidin-3, 5- diglucoside, cyanidin-3, 5- diglucoside, palmitic, stearic, linolenic, leucocyanidin and gallic acid.

Hence the objective of the present study is to assess the functional feed efficacy of *Saraca indica* supplemented diet on Mossambic tilapia(O.*mossambicus)* with emphasis on microbiological and bio chemical parameters.

MATERIALS AND METHODS

Experimental setup

Oreochromis mossambicus Juveniles were collected from Agency for Development of Aquaculture in Kerala (ADAK) at Varkala. The experiments were carried out in triplicate using glass tanks of 1000 L capacity with 20 fish per tank. Fish were allowed to starve for 24 hrs to recover from the handling stress prior to feeding, and then they were acclimatized in the laboratory for 15 days. Water quality parameters were maintained every day and monitored periodically, 50% water was renewed daily during removal of fecal materials and unused feed.

Methanolic extract of the *Saraca indica* was prepared as per the method outlined by Deshmukh & Borle (1974).To evaluate the functional feed efficacy of *Saraca indica* supplemented diet on O. mossambicus, the experimental fish were fed with medicated feed (basal feed + 0.8% methanolic extract of *Saraca indica* included diet) for two months. Simultaneously a set of control fish were also maintained and were given basal feed (Hardy *et al*., 1978). Both groups were fed @ 2% of their body weight twice daily. After the experimental period the fish were sacrificed and the tissues were processed as per standard protocol. Biochemical analysis of tissues such as muscle, liver, gill,gut. head kidney and skin were done. Total protein in the above tissues were assayed by Lowry's method using Bovine serum albumin as standard. The glycogen content in the tissue was estimated by Anthrone method following Sifter *et al* (1950) and the total Lipid content was estimated by Chloroform Methanol method ,and microbial analysis were done as per method out lined by .(Kannan *et al*., 2003)and species diversity (Jayasree *et al*., 2012)

RESULTS AND DISCUSSION

The results obtained are presented in Table 1 to 5. Table 1 represents the physic-chemical characters of culture water. The physic-chemical parameters measured were fluctuated slightly during the experimental period and the values were normal for fish rearing.

Table 1. Water quality parameters during the experimental period.

Water quality parameters	Control tank	Experimental tank
pH	6.7±0.15*	6.7±0.02
Temperature (°C)	29±0.14	29±0.13
Dissolved Oxygen (ppm)	6.5±0.4	6.1±0.4
Salinity (ppt)	2.5±0.02	2.5±0.12

* Mean±SE

Table 2 shows the microbial load in the culture tanks .Table 3 depicts the total bacterial load at gill, gut and skin of both control& treated *O. mossambicus* at two terminations. There is an increase in the total microbial load at the tissues of control, than treated further species level isolation shows that most of the isolated strains were of probiotic type in the treated. Similar results were obtained by sameeda.et.al(2011) in *O. mossambicus* (*Curcuma ameda*) and Jasmine *et al.* (2011) in *Macrobrachium rosenbergii* with *Ocimum sanctum*(0.25%).

Table 2. Microbial load of water in the culture Tanks

Sample	Total plate count (CFU/ml)	
	30days	60days
Control	7.83±0.12*	6.76±0.04
Treated	7.85±0.58	6.85±0.04

*Log transformed values n=6

Table 4 shows the main bacterial strains isolated from *O. mossambicus* after treatment with *Saraca indica*. The probiotic strains isolated from the Gill of treated fish after all terminations were *Lactobacillus* Type 1 &11,, Bacillus. Type 1 &11, Staphylococus, *Cytophaga Moraxella* and *Flavo bacterium* and they represent the normal micro flora in the typical fresh water environment. The probiotic strains isolated from the Gut of treated fish were Lactobacillus Type 1 &11,, *Bacillus*. Type 11, & 111. LAB isolates from fish gut can act as probiotics and can prevent colonization of gut by pathogenic bacteria (Ringo and Gatesoupe, 1998. The probiotic strains isolated from the skin of treated fish after all terminations were *Lactobacillus* Type 1 Bacillus. Type 1 & 11,Staphylococus Moraxella. Earlier work of Kuttanpilly *et al*., 2004 on *P. monodon* agrees with our findings, viz herbal diets prepared from *Adathoda vasika, Murraya koenigii, Ocimum basilicum, Psoralea cordifolia* and *Qurcus infectoria* can effectively suppress many bacterial strains affecting *P. monodon*.

Table 5 Bacterial isolates at the non specific immune sites of *O. mossambicus* (control) Pathogenic and opportunistic pathogens show a reduction in all tissues of the treated fish than control and positive indication of feed quality. In the present study the metabolic extract of

Table 3. Microbial load at certain tissues of *O. mossambicus* - Treated (Supplemented with *Saraca indica* included diet) & control (basal feed)

Sample	Gill CFU/ml		Gut CFU/ml		Skin CFU/ml	
	30days	60 days	30 days	60 days	30 days	60 days
Control	3.97±0.038	3.93±0.01	3.40±0.1*	3.8±0.02	4±0.5	4.5±0.54
Treated	3.47±0.006	3.51±0.54	3.131±0.1	3.11±0.50	3.54±0.21	3.13±0.02

*Log transformed values, n=6

Table 4. Bacterial isolates at the non specific immune sites of *O. mossambicus* supplemented with *Saraca indica*

Bacterial isolates	**1st termination**						**11nd termination**					
Sample	**Gill**		**Gut**		**Skin**		**Gill**		**Gut**		**Skin**	
Moraxella(gram-ve)	1	+	0	-	12	+	18	+	0	-	1	+
Staphylococcus(g+ve)	0	-	0	+	2	+	0	-	6	+	1	+
Micrococus(gram+ve)	0	-	0	-	0	-	0	-	0	-	0	-
Bacillus type1(g+ve)	4	+	0	+	1	+	4	+	5	+	1	+
Bacillus type11(g+ve)	0	-	2	+	1	+	0	-	0	-	0	--
Bacillus type11(g+ve)	8	+	0	-	0	-	0	-	6	+	2	+
Bacillus type1v(g+-ve)	0	-	0	-	0	-	0	-	0	-	0	-
Lacto bacillus type1,(g+ve)	114	+	18	+	44	+	4	+	11	+	3	+
Lactobacillus T.11g,+ve)	1	+	0	+	0	-	0	-	3	+	12	-
Flavobacterium (g-ve)	10	+	0	+	0	+	0	+	0	+	2	+
Cytophaga(gram-ve)	5	+	0	+	3	+	0	+	0	+	0	+

Table 5. Bacterial isolates at the non specific immune sites of *O. mossambicus* (control)

Bacterial isolates	After 30 days						After 60 days					
	Gill		Gut		Skin		Gill		Gut		Skin	
Staphylococcus(g+ve)	2	+	1	+	3	+	0	-	0	-	8	+
Micrococus(g+ve)	56	+	28	+	104	+	57	+	48	+	76	+
Bacillus type1(g+ve)	1	-	10	+	0	-	0	-	10	+	0	-
Bacillus type11(g+ve)	0	-	0	-	0	-	3	+	6	+	0	-
Bacillus type111(g+ve)	0	-	8	+	0	-	0	-	9	+	0	-
Bacillus type1V(g+ve)	0	-	1	+	0	-	0	-	1	+	2	+
Moraxella(g-ve)	76	+	49	+	86	+	44	+	0	-	0	-
Lactobacillustype1g+)	0	-	3	+	0	-	0	-	0	-	0	-

Table 6. Biochemical Analysis of Gill, Gut, Liver, Muscle and Skin of *O. mossambicus (*1) control (11)

SAMPLE	Control (mg%)			Treated (mg%)		
	Protein	Glycogen	Lipid	Protein	Glycogen	Lipid
Gill	5.53± 0.02	0,92±0.12	0.67±0.08	8.53± 0.02	0,122±0.12	2.92±0.12
Gut	5.10±0.21	0.68±0.07	0.52±0.23	9.52±0.24	0.052±0.23	2.76. ±0.83
Skin	12.80±0.11	0.87±0.02	0.97±0.02	18.52±08	0.52±0.33	4.10±0.23
Liver	5.54±0.62	9.4±0.12	6.07±0.14	11.52±0.13	7.52±0.11	4.52±0.40
Muscle	16.64±13	1.9±0.32	0.62±0.01	20.52±0.23	4.52±0.41	5.59±0.23

Saraca indica supplemented diet effectively controlled bacterial growth in the culture media and it also regulated the growth of Pathogenic & opportunistic bacteria at all nonspecific immune sites of *Oreochromis mossambicus* such as gill gut and skin. Hence present study can be concluded that 0.8% *Saraca indica* supplemented diet can be effectively used to control Pathogenic and opportunistic bacteria in the culture system. Table 6 shows the biochemical turnover of control and treated fish at different sites. The biochemical turnover as reflected by the protein, glycogen and lipid content of vital tissues gives an understanding of assimilation, utilisation and balance equation for these biomolecules.

CONCLUSIONS

With the emerging trend in aquaculture through increased productivity, intensification, integration, industrialization and diversification .the sector also poses major concerns with regard to environmental degradation and competition for environmental goods and services. Sustainable aquaculture would therefore need rational and responsible use of all the inputs including drugs, chemical and other pharmacological components which need to be regulated through proper guidelines and strict norms. Present study can be concluded by stating that methanolic extract of *Saraca indica* is quiet effective as an antimicrobial agent had improved biochemical turn over and nitrogen excretion efficacy,. The aquatic ecosystem can be effectively saved from the harmful effect of antibiotics used in the culture system.

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