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QUALITATIVE ANALYSIS OF GUT MICROFLORA IN CULTURED FISH ROGU *(Labeo rohita L.)* AFTER THE ANTIBIOTIC ADMINISTRATION

Zoology

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ABSTRACT

In the present study the Rogu fish (Labeo rohita L.) were purchased from Raja fish pond at valangaiman, Thiruvarur district, Tamil nadu, South India. Fish were treated Group I and Group II, Group I reared in antibiotic free fed. Group II was treated every day antibiotic mixed fed. In the study the effect of antibiotic on the growth performance of fish were analysed at various time interval (0, 7, 14, 21 and 28 days). Each treatment separately was analysis of bacterial population (Aerobic plate count) from fish intestinal, haematology parameters from fish blood and biochemical characterisers from fish tissue up to 30 days. Totally in the present study bacterial population were maximum level decreased in antibiotic mixed fed inoculated fish intestinal tract and the blood parameters such as WBC, RBC, Hemoglobin, Plates, Lymphocytes and Monocytes are slightly increased in treatment-II when compared than treatment-I. Maximum carbohydrate level presented in the Treatement-II at the same slightly increase the Treatment-I. Total protein were analyzed in two treatment, among this study maximum protein content were recorded in Treatment-II tissue of compared then Treatment-I. The lipid content also estimated in two treatments.

KEYWORDS

Rogu, Antibiotic, bacterial population

INTRODUCTION

Aquaculture is the fastest growing food-producing sector in the world with the greatest potential to meet the growing demand for food. In contrast to other animal by an enormous diversity of species raised both in natural and artificial systems. Commercial catfish culture has been increasingly gaining importance in India. Indigenous *Catfish shingi (Heteropneustes fossilis)* and magur *(Clarius batrachus)* are widely distributed freshwater fish species. Microorganisms are widely distributed in nature and are found mostly in natural water. In urban and densely populated rural areas, the microbiological quality of fresh water is frequently threatened by contamination with untreated domestic wastewater (Griesel and Jagals, 2002). The microorganisms influence the water quality and are closely associated with the fish physiology and diseases. Nutrients increase is readily incorporated with the microbial community and ultimately into the fish biomass. Usually, aquatic animal including fish takes a large number of bacteria through their food and drinking water which accumulate in their intestine.

Following the discovery of the growth promoting and disease fighting capabilities of antibiotics, fish farmers began using such drugs in feeds. Antibiotics routinely used for treatment of human infections are also used for animals, for therapy, prophylactic reasons or growth promotion. For the last named purpose, sub therapeutic doses of antibiotics usually have been used, and this has contributed to promoting resistance. These uses of antibiotics can also create antibiotic resistance in non-pathogenic bacteria, the resistance genes of which can be transferred to disease-causing bacteria, resulting in antibiotic-resistant infections for humans. The report from the invitational European Union conference on the digestive tract of adult marine fish has been reported to contain *Aeromonas, Alcaligenes*, *Alteromonas*, *Carnobacterium, Flavobacterium, Micrococcus, Phot obacterium, Pseudomonas, Staphylococcus and Vibrio, including V. iliopiscarius.*

Terminal restriction fragment length polymorphism data point to a greater diversity in the posterior compared to the anterior gut in large herbivorous fish, i.e., Kyphosus sydneyanus (Al-Harbi, and Uddin, 2005). Nutrition and feeding play a central role in sustainable aquaculture and therefore, feed resources as well as costs continue to dominate aquaculture needs. Feed accounts for 40-60% of the production costs in aquaculture, with protein sources accounting for a significant proportion of this cost (Fotedar, 2004). Hence, the present study was undertaken quantitative analysis of gut microflora in cultured fish the antibiotic admonition.

MATERIALS AND METHODS

Collection of fishes

The *Labeo rohita* L. fish was collected from the Raja fish pond at valangaiman, Thiruvarur district, Tamil nadu, South india.

Collection of Antibiotics

All the chemicals used in this project work were purchased from Himedia, Mumbai. The antibiotic sample, Streptomycin was used for the present experimental studies.

Maintenance of fish

Before experimentation, the fishes were acclimatized to laboratory conditions for 30 days during which they were regularly fed with oil-less groundnut cakes. The water used was changed twice a day and were fed with standard fish food. Salinity, temperature, pH and dissolved oxygen content of the medium were average range from 0.4 to 0.5 ppm, 28±2ºC, 7.4-7.8 and 1.4 ppm respectively.

Experimental Design

After acclimatization the test organisms were divided into two groups and named as group I to group II. Group I was considered as control and reared in antibiotic free fed. Group II was treated every day antibiotic mixed fed 1/10 W/W. After 7 days, each treatment separately was analysis of bacterial population (Aerobic plate count) from fish intestinal, haematology parameters from fish blood and biochemical characterisers from fish tissue up to 30 days.

Bacterial population (Aerobic plate count)

The treated *Labeo rohita* L fish intestinal samples were collected and homogenized and used as a sample for bacteria isolation. The homogenated sample was serially diluted from 10^{-1} to 10^{-7}. From the diluted sample take 0.1ml of sample from 10^{-4}, 10^{-5} and 10^{-6} and spread over on nutrient agar medium separately. One plate maintain as a control without sample. The plates were incubated at 37°C for 24 Hrs observe the bacterial colonies.

Haematological parameters

Five fishes from each treatment group (T1, T2) were randomly selected from treatment unit at the end of each 7 days upto 30 days. To bleed alive, Fish samples were anaesthetized with MS222 (buffered solution; 30mg/L). The wet body weight (g) and total length (cm) of samples were also recorded at the time of blood collection. Blood samples were drawn by direct puncturing the heart by using 1 ml hypodermic syringe (21 gauge), usually used in insulin administration. A special Attention was taken to inhibit the blood from coming in contact with water. A vial containing the anti-coagulant EDTA (Heparin sodium 1%) was used for blood cell studies (Remya, 2010). The collected blood samples were rapidly subjected to haematological laboratory for analysis. The blood indices; MCV-Mean corpuscular volume, MCH-Mean corpuscular haemoglobin, MCHC-Mean

corpuscular haemoglobin concentration, WBC-White blood cells, LYM-Lymphocytes, MON-Monocytes, GRA-Granulocytes, RBC-Red blood cells, HGB-Haemoglobin, RDW-Red blood cell distrib ution width, PLT-Platelets and MPV-Mean platelets volum ewere determined in all the groups by using Mythic 18 automatic hematology analyzer, Orphee, Switzerland.

Biochemical analysis

Protein was measured by the method of Lowry et al., (1951) with bovine serum albumin as the standard. Carbohydrates content was measured by Anthrone method (Hedge and Hofreiter, 1962). To estimation of lipid was analysed by Cox method (Cox et al., 1962).

Statistical analysis

The results obtained in the present investigation were subject to statistical analysis like standard deviation (SD) and students 'T' test by following the procedure given by Zar (1984).

RESULTS AND DISCUSSION

The present study quantitative analysis of gut microflora, growth gain and biochemical compounds were analyzed from cultured *Labeo rohita* L fish the antibiotic admonition. The *Labeo rohita* L growth rate weight was evaluated in the two treatment fish at different time interval (0, 7, 14. 21 and 28 days) (Fig-1). From the Table-1 results, maximum growth gain percentage recorded in Treatment 1(Normal fed + Antibiotic) compared than other treatments. At the same time no significant variation noted on treatment I and II. The Highest growth rate recorded at 30th days Treatment.

Labeo rohita L lengths also measured before and after feeding the results were presented in the Fig. 1. The fish maximum length increased in treatment-II at the same treatment-I slightly increased. In this experiment 28 day weight and length are highly increased compared than before Treatment days.

The treated *Labeo rohita* L fish's intestinal tract contain bacterial population level was estimated (Table – 1). The maximum level of bacterial population was present in treatment-I (127×10^{-6} CFU/g) when compared than treatment-II. Totally in the present study bacterial population were maximum level decreased in antibiotic mixed fed inoculated fish intestinal tract. The result of the present study showed variations in total bacterial load of intestine. The physiological effect of the bacterial flora of the GI tract is described as "Fermentation of non-digestible dietary residues and endogenous mucus: salvage of energy as short-chain fatty acids, production of vitamin K, absorption of ions; control of epithelial cell proliferation and differentiation; development and homoeostasis of the immune system and protection against pathogens (the barrier effect)". The host provides nutrients to bacteria and bacteria repay by providing a colonisation barrier (Guarner and Malagelada, 2003). The treated fish blood Haematology parameters were analyzed. The investigated results were presented in Table – 2. The blood parameters such as WBC, RBC, Hemoglobin, Plates, Lymphocytes and Monocytes are slightly increased in treat ment-II when compared than treatment-I.

From the table-3 showing maximum carbohydrate level presented in the Treatement-II at the same slightly increase the Treatment-I. In this estimation have high amount of carbohydrate present in the 28th day treatment fish compared than other days treated fish. Total protein were analyzed in two treatment, among this study maximum protein content were recorded in Treatment-II tissue of compared then Treatment-I. At the same time highest protein content were recorded at 28th day both fish sample (Table-3). Proteins are important biomolecules involved in a wide spectrum of cellular functions (Prasanth, 2006).

The lipid content also estimated in two treatments. In this experiments 28th day antibiotic mixed feeding fish present high amount lipid content (Table-3). From the Fig.-3 revealed maximum lipid content presented in the Treatment-II compared than Treatment-I. The decrease in tissue lipid and proteins might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of cell membranes, and cell organelles present in cytoplasm (Harper, 1983).

CONCLUSION

Superiority of antibiotics was well pronounced as it served the double role as direct feed to growing fishes and as direct manure for increasing growth of fish food, in view of the need of the organic aquaculture antibiotic could serve as a direct application feed for the fish forming ponds.

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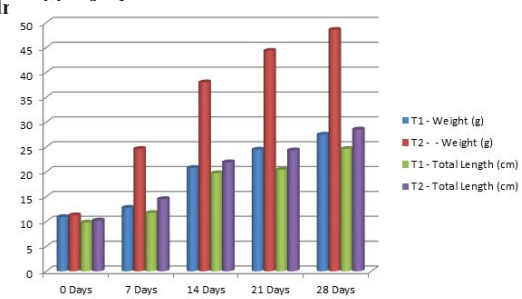
Fig. – 1 Growth parameters of *Labeo rohita L.* at antibiotic adr



T1 - Control and reared in antibiotic free fed
T2 - Treated every day antibiotic mixed fed

Table – 1 Bacterial Population of *Labeo rohita L.* at antibiotic admonition feed

S. No.	Different time interval	No. of CFU/g	
		T1	T2
1	0 Days	135×10-6	130×10-6
2	7 Days	168×10-6	65×10-6
3	14 Days	155×10-6	33×10-6
4	21 Days	170×10-6	TNLC
5	28 Days	127×10-6	TNLC

CFU - Colony Forming Unit
TNLC – Too Numerous Low count

T1 - Control and reared in antibiotic free fed
T2 - Treated every day antibiotic mixed fed

Table – 2 Haematology parameters of Labeo rohita L. at antibiotic admonition feed

S. No.	Haematology parameters	Different time interval									
		0 Days		7 Days		14 Days		21 Days		28 Days	
		T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
1	WBC (10^3/µl)	110.2±2.6	114.5±0.8	115.5±1.2	124.5±1.5	114.7±1.4	135.8±1.8	120.4±1.6	138.2±1.4	121.7±0.4	141.9±0.5
2	RBC (10^3/µl)	2.21±0.6	2.25±0.4	2.15±0.7	2.21±0.2	2.18±0.5	2.28±0.7	2.20±0.6	2.32±0.4	2.22±0.8	2.36±0.4
3	Hemoglobin (g/dl)	8.45±0.2	8.80±0.7	8.4±0.4	9.2±0.6	8.6±0.8	9.5±0.2	8.5±0.5	9.8±0.3	8.6±0.2	10.1±0.4
4	Platelets (10^3/µl)	160±10.2	165±05.6	168±09.1	175±07.8	168±06.2	178±10.2	170±08.7	182±09.2	168±04.5	185±07.5
5	Lymphocytes (10^3/µl)	100±05.4	105±08.0	108±04.2	105±05.6	108±07.3	108±08.7	110±04.0	112±05.0	118±07.2	125±06.0
6	Monocytes (10^3/µl)	6.4±0.4	6.8±0.9	6.4±0.7	6.2±0.5	6.6±0.7	6.5±0.5	6.5±0.2	6.8±0.4	6.6±0.3	7.1±0.5

Values are expressed Mean ± Standard deviation; n=3
T1 - Control and reared in antibiotic free fed
T2 - Treated every day antibiotic mixed fed

Table – 3 Biochemical analysis of *Labeo rohita L.* at antibiotic admonition feed

S. No.	Biochemical Parameters	Different time interval									
		0 Days		7 Days		14 Days		21 Days		28 Days	
		T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
1	Crude Protein	210.2 ±10.6	214.5±10.8	215.5±11.2	224.5±11.5	214.7±11.3	235.8±11.6	220.4±11.8	238.2±11.4	221.7±10.5	241.9±10.0
2	Carbohydrate	3.21± 0.5	3.25 ±0.8	3.15 ±0.6	3.21 ±0.7	3.18 ±0.9	3.28 ±0.2	3.20 ±0.6	3.32 ±0.8	3.22 ±0.4	3.36 ±0.6
3	Lipid	9.45± 0.2	8.80 ±0.5	8.4± 0.4	9.2± 0.6	8.7± 0.7	9.5± 0.2	8.9± 0.2	9.8± 0.3	8.6± 0.2	10.5 ±0.8

Values are expressed Mean ± Standard deviation; n=3

T1 - Control and reared in antibiotic free fed
T2 - Treated every day antibiotic mixed fed

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