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Biochemical Studies in Cat fish (*Clarious lazera*) Infected with *Flavobacterium columnare* and Treated with probiotic

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ABSTRACT

The present study was planned to investigate the effect of Flavobacterium columnare infection on some hematological, serum biochemical and pathological alterations of Clarious lazera. Forty five fish were divided into three equal groups. Fish of first group served as a control. Fish of group (2&3) were naturally infected by Flavobacterium columnare. Fish of group (3) were treated after 7 days of post-infection using the probiotic bacterium, Lactobacillus rhamonsus for 10 days. Sampling was done after 1 and 7 days of post-infection (gps 1 & 2) and 10 days of post-treatment (gps 1& 3). The results revealed a non significant changes in the hematological and the biochemical parameters after 1 day of infection, but after 7 days of post-infection and 10 days of post-treatment, a significant decrease in RBCs, Hb, PCV and significant increase in AST, ALT, urea, creatinine, sodium, potassium, cortisol, insulin and glucose were seen. Iron showed a significant decrease at the same period of sampling. The infected skin loses its natural sheen and a gray, white or yellowish margin surrounds the foal lesion. The mouth and inner walls of the oral cavity may be covered with a yellowish mucoid material. In scaly- less fishes, the lesions start with simple ulcers which predominately end with extensive saddleback-like ulcers exposing the underlying musculatures. Fin and gill rot is another lesion of the progressive infection in both scaly and scaly-less fishes. These reactions were evident, after 7 days of post-infection and the severity of the lesions were markedly decreased after 10 days of post-treatment. It could be concluded that, Flavobacterium columnare infections induced marked tissue alterations as well as some hematological and serum biochemical changes. Although probiotic treated the infected cases and allowed the regenerative processes but it does not progress the hematological and serum biochemical parameters.

Key words: Cat fish; *Flavobacterium columnare*; Biochemical changes

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INTRODUCTION

Columnaris disease is a predominant bacterial disease of both cultured and wild freshwater fish. Many commercially important fish species are susceptible to columnaris disease, such as (but not limited to) salmonids, eels, carp, goldfish, tilapia and channel catfish (1-3). This disease also poses a problem for many freshwater fish (4-5). F. columnare is mentioned in most books of tropical aquarium fish diseases as the cause of cotton-wool disease. Post (4) reported fish exhibiting greyish-white discoloration especially on the head and around the mouth. These may evolve into whitish filaments emerging from the lesions, hence the name mouth fungus. Although F. columnare is described as being an important pathogen (6-8), as far as we know, it has never been actually isolated from tropical aquarium fish, nor has its significance and pathogenicity been studied in any way.

F. columnare infections may result in skin lesions, fin erosion and gili necrosis, with a high degree of mortality, leading to severe economic losses (5).

The term probiotic was firstly used to denominate microorganisms that have effects on other microorganisms (9).

Probiotics can provide some solutions to this problem through different mechanisms or properties such as the production of inhibitory compounds such as bacteriocins, competition for adhesion sites with opportunistic or pathogen microorganisms, competition for nutrients with other bacteria or an improvement of the immune status (e.g. increase of production of immunoglobulins, acid phosphatase, antimicrobial peptides, improvement of cellular activities, etc.) [10-17].

Use of microbial probiotics to promote health maintenance and disease prevention and control is now widely accepted as the new ecofriendly alternative measures for sustainable aquaculture [18]

The present work aimed to study the effect of *F. columnare* on biochemical and skin disease clinicopathological findings of infected *cat fish* before and after treatment with probiotic

MATERIALS AND METHODS

Fish: Forty five fish *naturally infected* with *F. columnare* average body weight of 100-150 gm/fish were obtained from River Nile and transported to the laboratory and reared in 3 equal glass aquaria (115 liter capacity), fed a balanced ration and provided with continuous aireated and renued tap water.

Fish were kept one week for acclimatization and mean time subjected to mycological, bacteriological and pathological examinations.

The probiotic bacterium, Lactobacillus rhamonsus (ATCC 53103) was cultured in MRS broth at 26.8C for 48 h, centrifuged and washed with sterile PBS 2 times.

Bacterial pellets were measured in PBS and their densities were determined. Under sterile conditions, the bacteria were manually incorporated into commercial dry pellets at rates of 108 and 1010 CFU/g in feed for low and high LAB diets, respectively. Fish fed only commercial dry pellets served as a control. Fish were fed approximately 0.8% of body weight once a day. The probiotic groups ingested an average of 3.8×10^6 and 3.8×10^8 cells day^{-1}.

Experimental infection: Fish were divided into 3 equal groups. Fish of group (1) were kept without treatment to serve as a control. Fish of groups (2 and 3) were naturally infected by *F. columnare.* Fish of group (3) were treated after 7 days of infection using probiotic (2.9×10^6) for 10 days.

Sampling: Blood samples and tissue specimens were taken at first and seventh days of infection (gp2) and also after ten days of treatment (gp3). Sampling was also done at the same tome from control group (gp1). Blood samples were taken in heparinised microhematocrit tube and other tubes to be centrifuged at 3000 r. p. m. for 10 min. for serum separation. The serum stored at 20^oC until analysis.

Hematological examinations: The erythrocytic indices (RBCs, Hb, PCV and MCV) and reticulocytes were determined according to Schalm (19).

Serum biochemical analysis: Serum aspirateaminotransferase (AST) and alanin aminotransferase (ALT) also serum urea, creatinine and glucose were estimated using kits supplied from Biomerieux (France). Sodium and potassium were determined by flame photometer according the method described by silversmith (20). Serum cortisol level was determined using radio immunoassay technique (21). Insulin was estimated by radioimmunoassay using kits obtained from diagnostic products corporation (Los Angeles, USA). Iron was determined using atomic absorption according to Joseph and Roger (22).

RESULTS

F. columnare, after 1 of day post-infection, induced non significant changes in the hematological and serum biochemical parameters. A significant decrease in RBCs, Hb and PCV was observed in cat fish, after 7 days of post-infection and 10 days of post-treatment, while MCV and a period of reticulocytes showed a high significant decrease at the same period of sampling in comparison with control. A significant increase in AST, ALT, urea, creatinine, sodium, potassium, cortisol, insulin and glucose was noticed in cat fish after 7 days of post-infection by *F. columnare* and 10 days of post-treatment while iron showed a significant decrease at the same period of sampling in comparison with control.

Clinically, cat fi*sh* infected by *F. columnare showed* he infected skin loses its natural skin and a gray, white or yellowish margin surrounds the foal lesion. The mouth and inner walls of the oral cavity may be covered with a yellowish mucoid material. In scaly fish, the lesions start with simple ulcers which predominately end with extensive saddleback-like ulcers exposing the underlying musculatures. Fin and gill rot is another lesion of the progressive infection in both scaly and scaly fish.

Table 1: Effect of naturally infected fish with *F. columnare* on some hematological parameters of cat fish before and after treatment in comparison with control (Mean±SE)

Parameters	Control gp.	Infected gp.		Treated gp. (10 days P.T)
		1 day P.I.	7 days P.I.	
RBC_s (10^6/mm^3)	2.42	2.43	1.94	1.73
Hb (gm/dl)	8.03	8.21	8.29	7.63
PCV	19.03	18.01	16.01	15.01
MCV (FL)	37.03	34.28	29.10	28.01
Reticulocytes (%)	1.60	1.40	1.22	1.54

***Significant at P<0.01, P.I. = Post-infection, P.T. = Post-treatment, gp. = group*

Table 2: Effect of naturally infected fish with *F. columnare* on some serum biochemical parameters of cat fish before and after treatment in comparison with control (Mean±SE)

Parameters	Control gp.	Infected gp.		Treated gp. (10 days P.T)
		1 day P.I.	7 days P.I.	
AST (U/L)	80.00±0.64	80.00±0.15	125.00±0.38	127.00±0.57*
ALT (U/L)	20.00±0.15	22.00±0.17	32.00±0.17*	37.00±0.13*
Urea (mg%)	3.03±0.30	3.07±0.34	4.05±0.52*	4.03±0.81*
Creatinine (mg%)	0.61±0.20	0.63±0.32	0.83±0.21*	0.89±0.12*
Sodium (mfg/dl)	125.00±0.32	135.00±0.71	148.00±1.10*	155.90±1.72*
Potassium (mfg/dl)	3.00±0.21	3.20±0.39	5.62±0.72*	6.30±0.70*
Cortisol (µg/dl)	0.70±0.18	0.75±0.17	1.02±0.64*	1.01±0.70*
Insulin (µg/dl)	9.20±0.14	10.60±0.70	12.20±0.40*	11.90±0.60*
Glucose (mfg/dl)	60.30±0.34	63.80±0.70	80.00±0.73*	82.80±0.70*
Iron (mg/dl)	221.00±0.10	223.00±1.10	212.00±1.10*	194.00±1.14*

**Significant of p<0.01*

DISCUSSION

It is apparent that, Nile cat fish infected with *F. columnare* caused a significant increase in glucose and insulin levels only during 7 days of infection and 10 days of treatment probiotic. It is well known that, any stress factor such as handling, incubation, or anathesia have been shown to cause hyperglycemia followed by hyperinsulinemia [19].

The present work revealed that, serum glucose was elevated during 7 days of infection and 10 days of treatment. One consistent effect of cortisol was the reduction in the haemoglobin, PCV, RBCs and iron level as a result of decrease in appetite in the cat fish or more likely to be the direct of catabolic effect of cortisol on the fish (24).

The experiment showed that sodium (Na) and potassium (K) concentrations were significantly increased, this retention may be attributable to kidney impairment where the kidney is the normal passway for Na and K, this may explain the main cause for elevation of serum creatinine and urea in the treated groups which also microscopically exhibited vacuolar degeneration of renal tubules. This confirms the previous results recorded by Osfor *et al*. (25), Zaki *et al*. (26) and Abdel Aziz *et al*., (27). This led to temporal changes in plasma insulin concentration which did not mirror those for glucose. One of the reasons may be the high sensitivity to glucose of pancreatic cells producing

somatostatin which in turn inhibits insulin secretion during the initial period after *F. columnare* (28). *F. columnare* infection causes a significant increase of cortisol level which may be due to the activation of hypothalamus pituitary internal axis. These results coincide with those observed by Jauncey and Ross (29) and Zaki et al. (30), who stated that, hyphae of *F. columnare* may invade deep tissues of the fish and penetrate the vital organs as kidney, liver and even the central nervous system and eye.

Marked elevations were noticed in the activity of (AST) and (ALT). The liver is the primary organ of detoxification as well as a major site for detoxification reaction, therefore, a significant increase in liver *F. columnare* explanations for the presence of the *F. columnare* or its toxins in liver. This picture was confirmed histopathologically by the marked vacuolar degeneration of hepatocytes.

As primary pathogen for stressed fish, this is in agree with Zaki *et al*. (26) and Badran *et al*., (31). Who stated that hyphae of *F. columnare* may invade deep tissues of fish and penetrate the vital organs even the central nervous system.

The clinical signs and postmortem lesions that reported among infected cat fish were similar to those reported by Aly and Ashram (33) and Attia [34] and Ferguson [35].

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