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CORELLATION BETWEEN BACTERIOLOGICAL INFECTION AND CHEMICAL CHANGES IN FISH CONSTITUENTS IN BOHIRA PROVINCE

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ABSTRACT

This study was applied on 100 fish samples (50 of each , Tilapia and Mugil) either diseased or apparently healthy. The aim of this work is to throw a light on the relation between the bacterial infection and some chemical changes in tissues of the examined fish as an index to quality and freshnes of raw materials.

The results of bacteriological examination revealed that out of diseased tilapia(20) 39 bacterial isolates were recorded, but in apparently healthy tilapia(30)isolates were 41, while diseased Mugil (10) showed 13 bacterial isolates, and apparently healthy(40) was had 33 isolates. The rate of isolation was higher in the gills and viscera than the tissues as follow: Gills (80/50%), viscera(70/60%) and tissues(45/26.7%) in diseased/healthy tilapia respectively. In case of Mugil the corresponding ratio were 60/30%, 50/37.5% and20/15. Different microorganisms were isolated , Aeromonas hydrophila,Pseudomonas fluorescens,Vibrio spp.,E.coli, Streptococcus iniae, Staph. aureus and Clostridium perfringens at different percentages.

Three items were stastically calculated as chemical changes in this work , indicative to spoilage of the fish samples in comparison to control one. Thiobarbituric acid as mg malonaldhyde/ kg(TBA) was not significant ≤ (t.value 0.77) , while the FFA(free fatty acids) and Peroxide value, milliquivalent O2/kg (PV) appeared with significant values (6.38 and 21.1), respectively. On the other hand in mugil samples the three items of chemical examination appeared with significant values ≤ (8.31, 4.78 and 40.95 respectively).

INTRODUCTION

Fishes are important members of aquatic ecosystem and an important source of food for human. For many reasons, fish often used as test animals in aquatic environmental researches. Enviromental pollution represents a major proplem in both developed and undeveloped countries. Egypt is one country, which suffers from high biosphere pollution(air,soil, and water) Many ecological changes occur in water as a result of human activities, including agricultural, industrial and municipal wastes ***(Katz,et al.,1969)***. Bacterial diseases are responsible for heavy mortality in fish**(Robert,1978).**

Bacterial fish diseases and infections are very common in fish keeping and are probably one of the hardest health problems to deal with effectively. Pathogenic bacteria can spread disease throughout the fish's body if they are absorbed through the gills or gut, or gain entry via the skin. This is known as a **systemic infection**. Other bacterial infections cause localised surface disease such as fin rot and ulcers, however, if these are not resolved they can lead to a systemic infection. In general there are four types of bacterial infections that the hobbyist needs to be aware off.

Bacterial diseases are among the most important causes of losses among fish stocks. A full understanding of the aetiological agent, the pathogenesis, biochemistry, antigenicity, epizootiology and the inter-relationship of stress-related and environmental factors is essential for successful management and control. Clostridium perfringens may be a useful indicators of the presence of remote faecal poulltion in poulluted water or waste waters.

The initial quality of raw material; in terms of their freshness, microbiological, chemical quality and physical damage; is an important factor which influences the quality of the end product***(Berna and Sukran, 2004 and Nogueras et al., 2007).***

MATERIALS AND METHODS

Examined fish:-

A total of 100 fish samplesfish, 50 samples of each **Tilapia nilotica** and **Mugil cephalus** (Bory) either diseased or healthy fish were collected from different markets in Bohira province. All fishes were examined for skin affections.

Bacteriological examination:-

Samples were collected aseptically from gills, muscles, liver and intestine and inoculated onto different solid media, MacConky's agar and MackConky's+ ampicillin agar, nutrient agar and manitol salt agar then incubated aerobically for 24hrs at 37C, and also on thiosulphat citrat bile salt sucrose(T.C.B.S) agar and Loffeler's methylene blue semi solid tube agar which incubated anaerobically at 37C for 24 hrs for isolation of vibro and clostridia respectivly.

After recording cultural morphological characters , pigmentation,the purified colonies were picked up and heat fixed smear stained with Gram,s stain were done, The pure isolate was picked up and inoculated into semisold agar tube for determination motility and preservation till preformation of further identification.

On the other side, from suspected positive Loffeler's methyleneblue tube(decolourised), a loopfull was inculated onto the surface of 10% sheep blood agar with neomycin sulphate 75 mg/ml for the isolation of Clostridium. (***Koneman et al., 1988***)

. All the purified isolates were identified by studying of the morphlogical characteristic and biochemical activity described in the Bergey's Manual of Systematic Bacteriology ***(Holt, 1986).***

Chemical examination:

1-Determination of free fatty acids. F.F.A content was determined by titration with standard sod.hydroxide

(0.1N) according to the method described in A.O.C.S**(1980).**

2-Determination of peroxide value PV **:** The PV was determined according to the procedure described in A.O.C.S **(1980).** The results were calculated as milliequivalent to peroxide per Kg of tested samples.

3-Determination of thiobarbiturate acid value (TBA) was done according to the method outlined by **(Smith et al 2001) .**

Table(1)Rate of bacterial isolation from different organs of diseased and healthy Tilapia.

Site of isolation	Diseased(20)				Healthy(30)			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
Gills	16(6)*	80	4	20	15(4)	50	15	50
Tissues	9(3)	45	11	55	8(2)	26.70	22	73.3
Viscera	14(8)	70	6	30	18(9)	60	12	40

(N)* Number of mixed infectin.

Table(2) Rate of bacterial isolation from different organsof diseased and healthy Mugil

Site of isolation	Diseased(10)				Healthy(40)			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
Gills	6(2)*	60	4	40	12(2)	30	28	70
Tissues	2(1)	20	8	80	6(1)	15	34	85
Viscera	5(1)	50	5	50	15(3)	37.5	25	63.5

(N)* Mixed infection.

Table(3).Prevalence of different microorganisms isolated from Tilapia samples.

Isolated microorganism	Diseased (56)						Healthy (56)					
	Gills		Tissues		Viscera		Gills		Tissues		Viscera	
	No	%	No	%	No	%	No	%	No	%	No	%
Aeromonas hydrophila	4	7.2	3	5.4	6	10.8	3	5.4	2	3.6	6	10.8
Pseudomonas fluorescens	2	3.6	3	5.4	3	5.4	3	5.4	1	1.8	4	7.2
Vibrio Spp.	3	5.4	1	1.8	3	5.4	0	0	1	1.8	5	8.9
Streptococcus iniae	3	5.4	2	3.6	1	1.8	3	5.4	2	3.6	2	3.6
Staph. aureus	2	3.6	1	1.8	1	1.8	4	7.2	1	1.8	2	3.6
Escherichiacoli	5	8.9	2	3.6	2	3.6	2	3.6	2	3.6	4	7.2
Clostridium perfringens	3	5.4	0	0	6	10.8	4	7.2	1	1.8	4	7.2

Table(4) Prevalence of different microorganisms isolated from Mugil samples.

Isolated microorganism	Diseased (17)						Healthy (42)					
	Gills		Tissues		Viscera		Gills		Tissues		Viscera	
	No	%	No	%	No	%	No	%	No	%	No	%
Aeromonas hydrophila	1	6	0	0	2	12	2	4.8	3	7.2	4	9.6
Pseudomonas fluorescens	1	6	0	0	1	6	3	7.2	1	2.4	3	7.2
Vibrio Spp.	2	12	1	6	0	0	0	0	0	0	2	4.8
Streptococcus iniae	0	0	1	6	0	0	4	9.6	2	4.8	4	9.6
Staph. aureus	2	12	1	6	0	0	2	4.8	2	4.8	0	0
Escherichia coli	1	6	0	0	1	6	2	4.8	1	2.4	3	7.2
Clostridium perfringens	1	6	0	0	2	12	1	2.4	1	2.4	2	4.8
Total	8	48	3	18	6	36	14	33.3	10	24	18	43.2

Table (5): Statistical analysis of chemical examination of examined Tilapia samples .

Parameter	Condition	Minimum	Maximum	Mean±SEM	t. value
Thiobarbituric acid, mg malonaldehyde /Kg	Diseased	0.300	0.490	0.416±0.009	0.77ns
	control	0.411	0.434	0.423±0.002	
Free fatty acids, %oleic acid	Diseased	0.200	0.268	0.232±0.003	6.38***
	control	0.201	0.220	0.210±0.001	
Peroxide value, milliequivalent O2 /Kg	Diseased	1.340	2.680	2.032±0.082	21.1***
	control	0.271	0.350	0.290±0.004	

SEM, Standard error of mean, not significant (P>0.05), ****P<0.0001

Table(6): Statistical analysis of chemical examination of examined Mugil samples .

Parameter	Condition	Minimum	Maximum	Mean±SEM	t. value
Thiobarbituric acid, mg malonaldehyde /Kg	Diseased	0.700	0.963	0.816±0.016	8.31***
	Control	0.652	0.701	0.680±0.003	
Free fatty acids, %oleic acid	Diseased	0.268	0.385	0.298±0.004	4.78***
	control	0.250	0.289	0.274±0.002	
Peroxide value, milliequivalent O2 /Kg	Diseased	3.045	4.443	3.79+0.080	40.95***
	control	0.487	0.523	0.507±0.002	

SEM, Standard error of mean, ** P<0.0001

DISCUSSION

(Table 1) show that the diseased Tilapia samples(which had different lesions as red spots, swollen abdomen and dark gills) have a high rate of isolation than that of healthy one ,gills 80% /50%, tissues 45% / 26.7% and viscera 70% / 60%. On the other hand mixed infection appeared high in both gills and viscera than tissues samples either in diseased or healthy .The difference in the rats of isolate may be due to that most organisms are normal ihabitants and under any strees factors they change to pathogenic strains. Nearly ,this results agreed with **Ahmed et. al.,2005** who proved that total viable count of bacteria was high in gills and intestine. As for Mugil (Table 2) the rate of isolation was little than that of Tilapia where it was in gills 60% / 30%, tissues 20% / 15% and viscera 50% / 37.5% in both diseased and healthy Mugil. From the same table the gills and viscera had a high percentages than the tissues with generally little number of mixed infection.

(Table 3) reveales that there are 112 isolates recovered from diseased and healthy . These isolates were identified intoAeromonas, Pseudomonas, Streptococcus, Staphylococcus, E.coli and Clostridium with different percentages of 7.2, 3.6, 5.4, 5.4, 3.68.9 and 5.4 in gills in relation the total isolates respecively while in tissues they were 5.4, 5.4, 1.8, 1.8, 3.6 and zero. Regarding to viscera it appear that the Aeromonas and Clostridia were high(10.8%) in comparison to that of Pseudomonas., Vibrio, Streptococci., Staphylococci. And E. coli (5.4, 5.4, 1.8, 1.8 and 3.6% respectively). In healthy fish the ratio appeared at the same level of diseased fish but the number of samples were different(10 diseased to 40 healthy). These results nearly agree with ***Badran and Eissa(1991) and Aly (1994)*** who mentioned that Pseudomonas spp. was the main cause of septicaemia, while ***Okaeme(1989)*** isolated Ps. Flourescence and Ps. aeruginosa from affected Tilapia. The results partialy correspond to the study conducted by ***Eissa and Abd-Alla (1991), Eissa et al.,(1993), Aly(1994,), Eissa (1994), Plump(1994) and laila et al., a&b(2004)*** where Vibrio, Aeromonas ,Pseudomonas , Clostridia, Streptococcus, Staphylococcus and E. coli were dominant bacteria in Tilapia intestine. The presence of a high bacterial load in gills and intestine of fish might be due to high metabolic activity of fish associted with increased feeding rates at higher temperature (**AL-Harbi and Uddin 2005**) . Streptococcus sp. have been reported from diseased Tilapia (**Al-Harbi, 1994**).Moreover, these bacteria have been known to pass from fish to humans that have handled them (**Kohler,2000**). Once an outbreak is in progress, the Vibrio spp. in the environment rises dramatically, therefore increasing the chance that exposed fish will also become infected**, Shoemaker et al(2001), Al-Harbi, & Uddin and Cai et al.,2008.**

(Table 4) demonstrats that a total of 49 bacterial isolates were obtained, from 17 examined diseased samples(10) while in examined healthy samples(40) the recovered isolates were 42.Vibrio and Staph. aureus were 12% in gills,6% tissues and failed to be

isolated from the viscera. While closteridia,E.coli and pseudomonas were isolated at the same ratio of 6% from gills and viscera with the exception to clostridia was 12% and they were not isolated from tissues. On the other hand Aeromonas, Streptococcus,E.coli and Pseudomonas were the predominant isolates in healthy samples 4.8%, 9.6% ,4.8% 7.2% from gills and 9.6%, 9.6%,7.2 abd7.2% in viscera respectively. Vibrio was only isolated from viscera at 4.8% and Staph aureus also was isolated at 4.8% from gills and tissues. ***Austin and Austin (1987),*** and ***Moustafa et al.,(1990)*** studied the ability of Vibrio in apparently healthy Mugil. ***Apun, et al., (1999)*** recorded that a total of 16 bacterial species were recovered from the water samples and the various organs of the fish. The intestines of all the fish species harboured the most number of different bacterial species. No bacteria was found in the muscle of any of the fish .Also ***Shoemaker et al.(2001), Nirmala et al. (2005) , Al-Harbi and Uddin(2005) and Cai et al.,2008*** isolated the same microorganisms.

Chemical changes:

The bacteria in fish organs can affect the storage life and quality of the fishery products ***(Kaneko, 1971).*** Oxidative rancidity is a serious quality problem conforonting the fishery industry because the larg proportion of highly unsaturated fatty acids found in fish lipids supposed to the oxidative deterioratin which develop the off-flavour.

Table (5) revealed highly significant changes($P<0.0001$) in free fatty acid(FFA) and peroxide value(PV) but not significant in thiobarbituric acid(TBA), as an indicator of the chemical quality of examined fish. TBA, FFA and PV are used as chemical quality and freshness indix. On the other hand in (Table 6), TBA value as an index for lipid oxidation revealed highly significant changes among Mugil samples with mean value of 0.816, FFA 0.298 and PV 3.79. *(**Roldan et. al.,1985**)* mentioned that FFA content (expressed as % oleic acid) has been taken as an index of lipid quality determination. FFA have not been reported to directly cause quality defects, they enhance lipid oxidation and off- flavour development and indirectly cause textural changes by protien denaturation(***Shewfell, 1981***). ***Prieto et al., 2007*** studied the effect of acute exposure to toxic cyanobacterial material containing MCs on antioxidant enzymes and lipid peroxidation has been studied in liver, kidney and gills of **Tilapia** fish (**Oreochromis niloticus**)

The increasing of TBA values may be attributed to deterioration of lipid and degradation of primary oxidation products(hydroperoxides), since these products are unstable and degraded to secondary oxidation products (malonaldhyde)> But, the decremental rat of TBA value is due to theinteraction of the decomposition products of protien with malonaldhyde to give tertiany products*(**Ayensa et. al., 1993 and Undeland 2001**)*.

TBA is a common assay used to follow lipid oxidation in food stuffs***(Ladikos& Lougovois, 1999)*** based on spectrophotometric determination of malondialdhyde. Recently, the presence of aldehyde in ffds, has attracted attention because of their effect on food quality and safty. In

addition, an unsaturated aldhyde like malonaldhyde ***(Saeed& Howell, 2002)*** extracted from fish muscle, is a well known lipid peroxidation products, while other unsaturated aldheyde, which can be formed from the breakdown of hydroperoxides, like glutaldhyde, need more investigation.

Water pollution, lack of sanitation and the inadequat hygienic measures on dealing with fish frome catching till marketing were found to be the major contributing causes, which alter hygenic quality of fish ***(Kirbry et. al., 2003).*** So, it is recommended to adopt hygienic measures during catching, handling, transportation and marketing of fish . **Conclution**

In present study, isolation of A. hydrophila, E. coli,Staphylococcus sp., Streptococcus sp., Closteridia perferngins and Vibrio spp. which are facultative pathogens or agents of food poisoning, is of importance. The present results indicated that the commensal bacterial flora included facultative pathogens which under conditions of stress could give rise to fish epizootics.This information will help in controlling the storage life quality of the fishery products.

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