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Stress Related Enterocci Effect on Cultured Oreochromis Niloticus and its Relations to its Economic Losses

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Abstract – **The effect of bacteria varies from that of primary pathogen to that, the secondary invader in the presence of other disease agents, they may also serve as a stress factors and predispose fish to other diseases.**

Therefore, the present study was carried-out to investigate the clinical signs and P.M. lesions and isolation and identification of the causative agent.

***Streptococcosis* is a bacterial infection among fresh water reared in aquaculture especially in the spring and summer months.**

The most important clinical signs and Postmortem lesions of Streptococcus infection, the Streptococcosis diseases was also known as "Pop-eye" since one of the most characteristic symptoms were the accumulation of mucopurulent exudate around the eye. The external clinical signs varied among species of infected fish.

The level of Total protein, albumin and albumin / globulin ratio showed a higher level in control saline groupn followed by the control bacteria groups. And the lowest level observed in the groups treated with ammonium choloride, lime and low oxygen levl treated groups and the groups treated with S. pnumoniae showed lower levels of the protein parameters, followed by S. iniae and S. fecalis.

The antibody titer level increased from 1st to 4th week. The higher level of antibody titer observed in the control bacteria group and control saline group and the lowest level observed in the ammonium choloride, then lime and low oxygen treated group.

The groups treated with S. pnumoniae showed lower level of antibody titer, followed by S. iniae and S. fecalis. The higher mortality level observed in control bacteria group followed by control saline group, while the lowest mortality level observed in ammonium chloride treated group, followed by low oxygen treated group and lime treated group, respectively. Also, the mortality level observed in groups treated with S. pnumoniae of higher mortality level, followed by S. iniae and the least level observed in the groups treated with S. fecalis.

The higher RLP % observed in control bacteria group treated with S. pnumoniae and the least level observed low oxygen level treated group with S. iniae.

Meanwhile the other groups treated with S. pnumoniae, S. iniae and S. fecalis achived medium RLP %. Except the lim treated groups that achieved the lower RLP level.

The most important histologic results due Streptococci infection were, Internally, the abdominal cavity usually contained variable amounts of purulent exudate and/or blood. A yellowish exudate often covered the peritoneum and the epicardium, and was also found in the cranial cavity. In addition, haemorrhages were also observed in muscles, spleen, liver and kidney.

The liver showed vacuolar degeneration and necrosis of most hepatic cells and pancreatic acinar cellsm, liver showed wide spread vacuolar degeneration, the spleen showed focal lymphoid depletion with hemolyses of erythrocytes, als the melanomacrophages were degenerated and necrotic.

Also our results indicated that, the higher weight losses due to dead fish at different treatments observed in the group infected with S. pnumoniae control bacteria group (1.17 Kg), S. faecalis low O2 group (1.04 Kg), S. pnumoniae lime (1.00 /Kg) and its economic losses were 11.70, 10.40 and 10 LE/30 cultured O. niloticus for the same groups respectively, and for each 100 O. niloticus fish the economic losses reached to 36.74, 34.37 and 33.40LE/100 fish for the previous groups, respectively.

Keyword – **Stress, Enterocci, Cultured Oreochromis Niloticus, Amm, Chloride and Oxygen Level, Economic Losses, Economic Returns.**

I. INTRODUCTION

Fish is among the most important sources of protein to human consumption, thus the study of the signs and lesions, induced by fish diseases, helps the protection in our national economy. (*Eyngor et al., 2008).*

Streptococci cause a significant economic losses to the world aquaculture industry *(Klesius et al., 1997)* . These have been estimated to exceed U.S $ 150 million annually (Shoemaker and Klesius, 1997).

The most susceptible fish species to Streptococcal infection were, Red drum fish (SciaenopsOcellatus); *Locke et al. (2007)*; Rainbow trout (Onchrhynchus mykiss and Onchrhynchus kisutch); *Klesius et al. (1997);* Gilthead Seabream (Sparus auratus), European seabuss (Dicentrachus labrax) and Spine foot (Siganus rivulatus) fish *Bromoage et al. (1999),* Barramundi (Lates calcaufer) fish *Eldar et al. (1999)*; rabbit fish (Siganus canaliculatus) *Yuasa et al. (1999);* and Ornamental fish *Locke et al. (2007)*

The aim of the present work is to, estimate the incidence of the effect of stress on pathogenicity of different strains of Streptococci in Oreochromis niloticus via its effect on blood serum protein constituents (albumin, globulin and total proteins), and serum enzyme, observe the histopathological alterations in different organs of fish experimentally infected with Streptococci, determination of the most important methods for prevention of Streptococcus in cultured fish through antibody titration and RLP and also determination of the economic losses resulted from enterococci especially streptococci.

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II. Materials and Methods

3.1. Materials:

Fish for experimental infection:

390 healthy fish (*O. niloticus*) were used for chronic infection. Then we take the three strains *(S. Fecalis, S. Iniai and S. Pneumonae)* to make chronic infection, in which we injected the pellets with different types of stress (Ammonium chloride, Lime Ca-O and Low O2 level) in different groups as follow :

Table 1: Show the design of experiment :

Type of strain	Type of injection	No. of fish	Total no. of fish
Amm. chloride	S. Fecalis	30	390
	S. Iniai	30	
	S. Pneumonae	30	
Lime (Ca-O)	S. Fecalis	30	
	S. Iniai	30	
	S. Pneumonae	30	
low O_2 level	S. Fecalis	30	
	S. Iniai	30	
	S. Pneumonae	30	
Control bacteria	S. Fecalis	30	
	S. Iniai	30	
	S. Pneumonae	30	
Control saline	saline	30	

Then we observed the fish for 6 days and take blood samples every 48 hours for four weeks until end of the experiment.

Hematological investigations:

Clinico-biochemical analysis:

1) Determination of serum total protein:

Serum total protein was determination according to *Doumas et al. (1981)* using commercial kits produced by Pasteur Lab.

Determination of serum albumin:

Serum albumin was determined according to *Reinhold (1953)*

Determination of serum globulin :

According to *(Coles, 1974).*

Antibody titration against Streptococcus species bacterin:

The remainder 160 fish of *O. niloticus* from chronic experiment were used for determination of antibody titration as schedule mention in the following table (8).

Table 2: Show the design of experiment of antibody titration

Type of strain	Type of injection	No. of fish	Total no. of fish
Amm. chloride	S. Fecalis	10	160
	S. Iniai	10	
	S. Pneumonae	10	
Lime (Ca-O)	S. Fecalis	10	
	S. Iniai	10	
	S. Pneumonae	10	
low O_2 level	S. Fecalis	10	
	S. Iniai	10	
	S. Pneumonae	10	
Control bacteria	S. Fecalis	10	
	S. Iniai	10	
	S. Pneumonae	10	
Control +ve	Vaccine of S. Fecalis only	10	
	Vaccine of S. Iniai only	10	
	Vaccine of S. Pneumonae only	10	
Control -ve	Not vaccinated	10	

Evaluation of potency of prepared vaccine against Streptococcus species:

Bacterin preparation:

Streptococcus strain isolates were used in the bacterin preparation according to the method described by *Sakai et al. (1984).*

Challenge test:

After 28 days both of injected with bacterin and control groups were injected with 0.2 ml of virulent strain of *Streptococcus species* previously adjusted to 6 X 10^8 cells/ml.

$$RLP = 1 - RLP = 1 - \frac{\text{Mortality of vaccinated fish}}{\text{Mortality of control}} \times 100$$

According to *Newman and Majnarich (1982).*

Histopathological examination:

Samples were taken from affected organs (liver, spleen, kidney), from naturally and experimentally infected fish with *Streptococcus species* for histopathological examination. According to the method described by *Roberts (1978).*

Economic losses:

The economic losses of the fish due to exposure to Enterococci infection were determined from dead fish, weight of dead fish and the losses in return due to dead fish according to the following equations *(Atallah and El-Banna, 2005).*

a- Weight of dead fish = Number of dead fish X Average weight of the fish (gm).

b- Losses in returns (L.E) = Weight of the fish (Kg) X Price of Kg fish (L.E).

Statistical analysis The hematological, biochemical and economical data was statistically analysed for the effects of groups (type of enterococci infection) using two-way ANOVA that was run using the computer package of the Statistical Analysis System *(SAS, 2001).* Means were separated using least square means of the same program.

IV. Results

I. Clinical signs and postmortem findings of Streptococci infection:

The most important clinical signs and Postmortem lesions of Streptococcus infection, the Streptococcosis diseases was also known as “Pop-eye” since one of the most characteristic symptoms were the accumulation of mucopurulent exudate around the eye. The external clinical signs varied among species of infected fish.

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Fig.1
Oreochromis niloticus experimentally infected with Streptococcus inai with amm. Chloride showing loss of scales, excessive mucus allover the body surfaces with hemorrhagic area especially under pectoral fin

Fig.2
Oreochromis niloticus experimentally infected with Streptococcus ina with lime showing tail rot and darkening of the dorsal surface with slight congestion of the head .

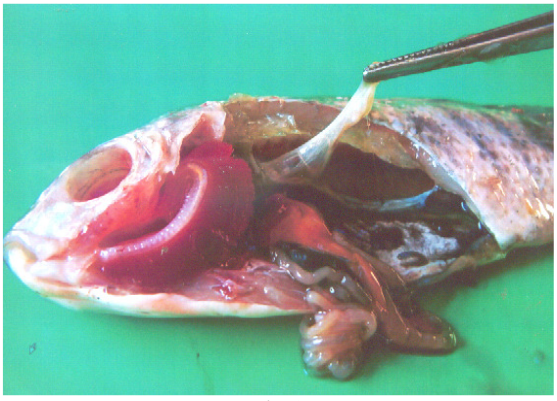

Fig.3
Oreochromis niloticus experimentally infected with Streptococcus pneumoni with lime showing hemorrhage and congestion of the internal organs

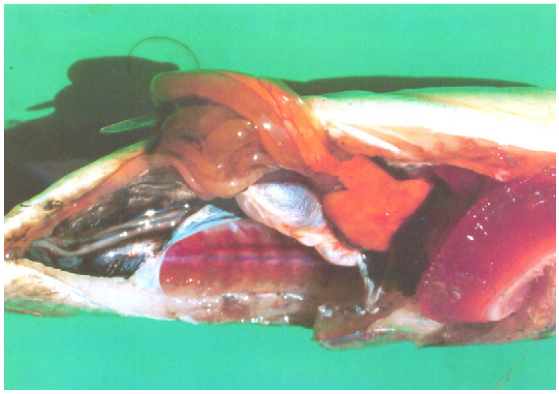

Fig.4
Oreochromis niloticus experimentally infected with *Streptococcus inai* with low O_2 showing hemorrhage and congestion of the gills and all internal organs with generalized septicemia

II. Results of haematological studies:

Results of total protein, Albumin, Globulin and Albumin/Globulin ratio:

Table (3) explains the level of total protein, Albumin, Globulin and Albumin/Globulin ratio (Serum proteins). The level of Total protein, albumin and albumin / globulin ratio showed a higher level in control saline groupn followed by the control bacteria groups. And the lowest level observed in the groups treated with ammonium choloride, lime and low oxygen levl treated groups and the groups treated with S. pnumoniae showed lower levels of the protein parameters, followed by S. iniae and S. fecalis.

Table 3: T. protein, albumin, globulin and albumin/globulin ratio in different groups among different days of experiment

Days	Type of strain	Type of injection	T. Prot.	Albumin	Globulin	Albumin globulin ratio
			Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
1st Day	Amm. chloride	S. Fecalis	4.4±0.33D	2.00±0.33B	2.4±0.04A	0.83±0.03G
		S. Iniai	3.50±0.33E	1.9±0.58B	1.6±0.06C	1.19±0.01E
		S. Pneumonae	4.00±0.33E	2.00±1.45C	2±0.02B	1±0.01F
	Lime (Ca-O)	S. Fecalis	4.00±0.33D	2.00±0.58D	2±0.02B	1±0.01F
		S. Iniai	4.2±0.33B	1.8±0.33B	2.4±0.03A	0.75±0.05I
		S. Pneumonae	4.1±0.58A	1.9±0.88B	2.2±0.03AB	0.86±0.06GH
	low O2 level	S. Fecalis	3.8±0.88C	1.8±0.58C	2±0.01B	0.9±0.02G
		S. Iniai	3.8±0.58C	1.9±0.33B	1.9±0.09B	1±0.01F
		S. Pneumonae	4.2±0.67E	1.9±0.58A	2.3±0.03AB	0.83±0.03HG
	Control	S. Fecalis	4.3±0.33B	2.7±0.33D	1.6±0.03C	1.69±0.06B

	bacteria	S. Iniai	4.50±0.58B	2.5±0.33A	2±0.02B	1.25±0.02D
		S. Pneumonae	4.50±0.33C	2.6±0.88B	1.9±0.03B	1.37±0.03C
	Control saline	saline	4.8±0.33C	3.00±0.58A	1.8±0.02B	1.67±0.01B
		Saline	4.6±0.33C	3.00±0.67C	1.6±0.03C	1.88±0.08A
2nd Day	Amm. chloride	S. Fecalis	4.67±0.33E	2.00±0.58D	2.67±0.07A	0.75±0.05K
		S. Iniai	4.33±0.33D	2.67±0.33E	1.66±0.06E	1.61±0.01C
		S. Pneumonae	4.00±0.58B	2.00±0.58C	2±0.02C	1±0.01H
	Lime (Ca-O)	S. Fecalis	4.50±0.58A	1.8±1.15A	2.7±0.07A	0.67±0.07L
		S. Iniai	4.00±0.58B	2.1±1.15B	1.9±0.03D	1.11±0.01G
		S. Pneumonae	4.33±0.88B	2.00±1.73B	2.33±0.03B	0.86±0.06J
	low O2 level	S. Fecalis	4.67±0.33B	2.00±0.58C	2.67±0.06A	0.75±0.05K
		S. Iniai	4.33±0.33C	2.00±0.33C	2.33±0.03B	0.86±0.06J
		S. Pneumonae	4. 1±0.33B	2.00±0.33AB	2.1±0.02C	0.95±0.05I
	Control bacteria	S. Fecalis	4.50±0.58A	2.50±0.58A	2±0.01D	1.25±0.02D
		S. Iniai	4. 6±0.58B	2.50±0.88C	2.1±0.02C	1.19±0.05E
		S. Pneumonae	4. 7±0.33C	2.50±0.33D	2.2±0.03C	1.14±0.04F
	Control saline	saline	5.00±0.58B	3.2±0.58C	1.8±0.02E	1.78±0.07B
		Saline	4. 7±0.58A	3.2±0.33C	1.5±0.01F	2.13±0.03A
3rd Day	Amm. chloride	S. Fecalis	4.1±0.33B	1.9±0.58B	2.2±0.02AB	0.86±0.06E
		S. Iniai	4.00±0.58B	2.1±1.45C	1.9±0.02B	1.11±0.01C
		S. Pneumonae	3.50±1.45C	1.8±0.58D	1.7±0.03C	1.06±0.06D
	Lime (Ca-O)	S. Fecalis	4.1±0.58D	2.2±0.33B	1.9±0.01B	1.16±0.06C
		S. Iniai	4.00±0.33B	1.9±0.88B	2.1±0.02AB	0.9±0.01E
		S. Pneumonae	4.00±0.88B	2.1±0.58C	1.9±0.03BC	1.11±0.01C
	low O2 level	S. Fecalis	4.2±0.58C	1.8±0.33B	2.4±0.04A	0.75±0.05F
		S. Iniai	4.00±0.33B	1.7±0.58A	2.3±0.03AB	0.74±0.02F
		S. Pneumonae	4.1±0.58A	2.00±0.33D	2.1±0.02B	0.95±0.05D
	Control bacteria	S. Fecalis	4.50±0.33D	2.50±0.33A	2±0.02AB	1.25±0.02B
		S. Iniai	4.7±0.33A	2.8±0.88B	1.9±0.01B	1.47±0.04A
		S. Pneumonae	4.8±0.88B	2.6±0.58A	2.2±0.02AB	1.18±0.01B
	Control saline	saline	5.00±0.58A	3.00±0.67C	2±0.01AB	1.5±0.05A
		Saline	5.00±0.67C	3.00±0.58A	2±0.03AB	1.5±0.01A

For each day: Means within the same column of different litters are significantly different at ($P < 0.01$)

III. Antibody titers:

Table (4) showed that, the antibody titer level increased from 1st to 4th week. The higher level of antibody titer observed in the control bacteria group and control saline group and the lowest level observed in the ammonium choloride, then lime and low oxygen treated group.

The groups treated with S. pnumoniae showed lower level of antibody titer, followed by S. iniae and S. fecalis.

IV. Mortality level, Relative level of protection (R.L.P):

Table (5) explain the higher mortality level observed in control bacteria group followed by control saline group, while the lowest mortality level observed in ammonium chloride treated group, followed by low oxygen treated group and lime treated group, respectively. Also, the mortality level observed in groups treated with S. pnumoniae of higher mortality level, followed by S. iniae and the least level observed in the groups treated with S. fecalis.

The higher RLP% observed in control bacteria group treated with S. pnumoniae and the least level observed low oxygen level treated group with S. iniae.

Meanwhile the other groups treated with S. pnumoniae, S. iniae and S. fecalis achived medium RLP %. Except the lim treated groups that achieved the lower RLP level.

Table 4: Antibody titer in different treated groups among different weeks :

Type of strain	Type of injection	1st week	2nd week	3rd week	4th week
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Amm. chloride	S. Fecalis	3.23±0.09B	3.00±0.58A	3.1±0.33B	3.9±0.58B
	S. Iniai	3.30±0.06B	3.00±0.58AB	2.00±0.58B	1.1±1.45C
	S. Pneumonae	2.17±0.03C	3.33±0.33B	3.50±1.45C	3.8±0.58D

Lime (Ca-O)	S. Fecalis	3.13±0.03C	2.67±0.33C	2.1±0.58D	3.2±0.33B
	S. Iniai	2.63±0.03A	2.33±0.33E	3.00±0.33B	2.9±0.88B
	S. Pneumonae	2.30±0.06B	2.33±0.33C	2.00±0.88B	1.1±0.58C
Low O2 level	S. Fecalis	2.50±0.06C	3.67±0.33B	2.2±0.58C	2.8±0.33B
	S. Iniai	3.53±0.09C	2.67±0.33AB	2.00±0.33B	4.7±0.58A
	S. Pneumonae	2.77±0.09A	2.00±1.15D	3.1±0.58A	2.00±0.33D
Control bacteria	S. Fecalis	3.43±0.03D	4.33±0.33A	4.50±0.33D	4.50±0.33A
	S. Iniai	4.20±0.06E	3.67±0.33B	4.7±0.33A	4.8±0.88B
	S. Pneumonae	3.53±0.03C	3.33±0.33A	3.8±0.88B	4.6±0.58A
Control saline	saline	5.77±0.03AB	5.33±0.33A	6.00±0.58A	7.00±0.67C
	Saline	4.87±0.03A	6.67±0.33B	5.00±0.67C	5.00±0.58A

Means within the same column of different litters are significantly different at (P < 0.01)

Table 5: RLP among different days of experiment

Type of strain	**Type of injection**	**No. of mort.**	**R.L.P.**	**% R.L.P.**
		Mean ± SE	**Mean ± SE**	**Mean ± SE**
Amm. chloride	S. Fecalis	7.67±0.33E	2.00±0.58D	1.33±0.33C
	S. Iniai	6.33±0.33D	2.67±0.33E	1.33±0.33C
	S. Pneumonae	8.00±0.58B	2.00±0.58C	0.67±0.33D
Lime (Ca-O)	S. Fecalis	7.50±0.58A	1.8±1.15A	0.33±0.33C
	S. Iniai	7.00±0.58B	2.1±1.15B	0.33±0.33C
	S. Pneumonae	8.33±0.88B	2.00±1.73B	1.33±0.33C
low O2 level	S. Fecalis	8.67±0.33B	2.00±0.58C	1.33±1.45A
	S. Iniai	7.33±0.33C	2.00±0.33C	0.00±0.58B
	S. Pneumonae	6. 1±0.33B	2.00±0.33AB	0.33±0.33C
Control bacteria	S. Fecalis	2.50±0.58A	2.50±0.58A	1.33±0.33B
	S. Iniai	2. 6±0.58B	2.50±0.88C	1.33±0.33B
	S. Pneumonae	9. 7±0.33C	2.50±0.33D	2.00±0.58C
Control saline	Saline	9.00±0.58B	3.2±0.58C	1.33±0.33B

Means within the same column of different litters are significantly different at (P < 0.01)

V. The most important Histopathologic results indicated that:

The most important histologic results due Streptococci infection were, Internally, the abdominal cavity usually contained variable amounts of purulent exudate and/or blood. A yellowish exudate often covered the peritoneum and the epicardium, and was also found in the cranial cavity. In addition, haemorrhages were also observed in muscles, spleen, liver and kidney.

The liver showed vacuolar degeneration and necrosis of most hepatic cells and pancreatic acinar cellsm, liver showed wide spread vacuolar degeneration, the spleen showed focal lymphoid depletion with hemolyses of erythrocytes, also the melanomacrophages were degenerated and necrotic.

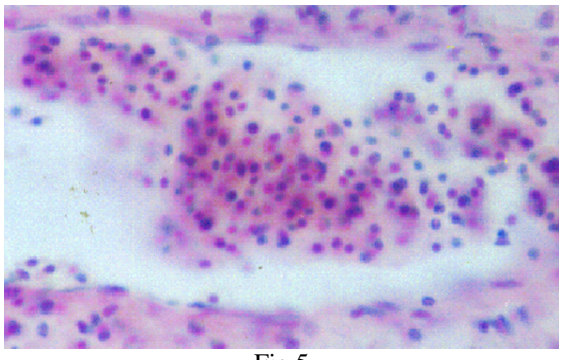

Fig.5
Liver of *O. niloticus* exposed Streptococcus inai showing vacuolar and hydropic degenerations of the hepatic cells. H & E x 150

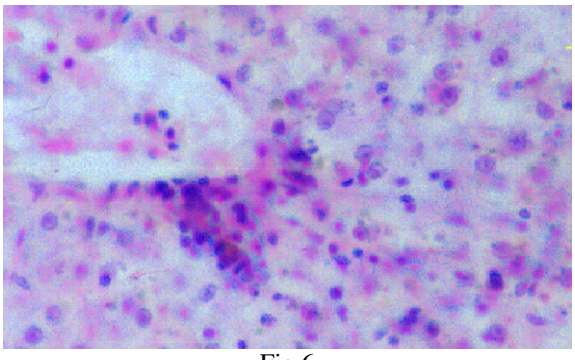

Fig.6
Liver of *O. niloticus* exposed to Streptococcus inai showing congestion of blood vessels.
H & E x 150

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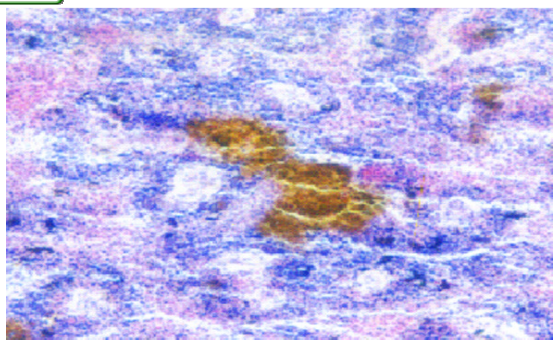

Fig.7
Spleen of *O. niloticus* exposed to Streptococcus fecalis showing numerous melanomacrophage centers.
H & E x 150

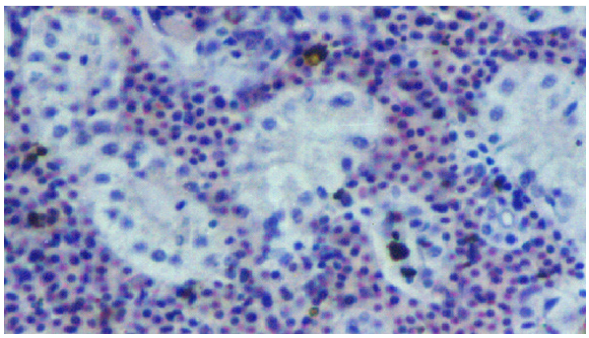

Fig.8
Kidney of *O. niloticus* exposed to Streptococcus fecalis showing degenerative change of the tubular epithelia and activated hemopoietic elements.
H & E x 600

VI. Economic losses of enterococci infected to O. niloticus:

Table 6) cleared the economic losses resulted from infection of the O. niloticus with different enterococci under different stress conditions (Amm chloride, Lime, low O2, control bacteria and control saline).

The higher weight losses due to dead fish at different treatments observed in the group infected with S. pnumoniae control bacteria group(1.17 Kg), S. faecalis low O2 group (1.04 Kg), S. pnumoniae lime (1.00 /Kg) and its economic losses were 11.70, 10.40 and 10 LE/30 cultured O. niloticus for the same groups respectively, and for each 100 O. niloticus fish the economic losses reached to 36.74, 34.37 and 33.40LE/100 fish for the previous groups, respectively.

Table 6: Economic loses resulted from enterococci infection in cultured O. niloticus .

Type of strain	Type of injection	No. of mort.	Weight of the dead fish/Kg	Return losses/30 fish/LE	Return losses/100 fish/LE
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Amm. chloride	S. Fecalis	7.67±0.33E	0.93±0.03B	9.30±0.40B	40.36±4.13B
	S. Iniai	6.33±0.33D	0.76±0.06C	7.60±0.50C	32.98±3.14C
	S. Pneumonae	8.00±0.58B	0.96±0.06B	9.60±0.70B	32.06±3.16B
Lime (Ca-O)	S. Fecalis	7.50±0.58A	0.9±0.04B	9.00±0.50B	30.06±3.14B
	S. Iniai	7.00±0.58B	0.84±0.04AB	8.40±0.50B	28.05±3.17B
	S. Pneumonae	8.33±0.88B	1.00±0.02A	10±0.10A	33.40±3.14A
low O_2 level	S. Fecalis	8.67±0.33B	1.04±0.04A	10.40±0.14A	34.37±4.17A
	S. Iniai	7.33±0.33C	0.88±0.03AB	8.80±0.13B	29.39±4.19B
	S. Pneumonae	6. 1±0.33B	0.73±0.03C	7.30±0.13C	24.38±4.14C
Control bacteria	S. Fecalis	2.50±0.58A	0.30±0.01D	3.00±0.11D	10.02±3.16D
	S. Iniai	2. 6±0.58B	0.31±0.01D	3.10±0.11D	10.35±3.17D
	S. Pneumonae	9. 7±0.33C	1.17±0.02A	11.70±0.22A	36.74±3.13A
Control saline	Saline	9.00±0.58B	1.08±0.02A	10.80±0.22A	36.07±3.14A

Means within the same column of different litters are significantly different at ($P < 0.01$)
Price of Kg fish in whole sale market = 10 LE; Weight of the dea fish = 120 gm

V. Discussion

The effect of bacteria varies from that of primary pathogen to that, the secondary invader in the presence of other disease agents, they may also serve as a stress factors and predispose fish to other diseases *(Badran and Eissa, 1991).*

Regarding to the experimental infection of the isolated Streptococcus, the present study showed that the same clinical signs, post-mortem and macroscopic examination, changes were in the form of tail rot haemorrhage of caudal peduncle with scale loss and absence of eye with bloody fluid in ocular cavity in advanced stage, eroded muscles of abdominal region at the 2nd week of experiment especially in groups infected with *Streptococcus*. Moreover the PM lesions were congetion of internal organs.

The successful reproduction of the disease experimentally leaves no doubt about the pathogenicity of isolated *Streptococcus Sp.* To infected fish. The fact that infection could occur following the presence in water contaminated with more than one species from *Streptococcus* confirms the invasive character of the organism. These results reported by *Khalil (2000).*

The Haematological examinations indicated that, The level of Total protein, albumin and albumin / globulin ratio showed a higher level in control saline groupn

followed by the control bacteria groups. And the lowest level observed in the groups treated with ammonium choloride, lime and low oxygen levl treated groups and the groups treated with S. pnumoniae showed lower levels of the protein parameters, followed by S. iniae and S. fecalis. This results attributed the degenerative changes that occur in the liver and heart *Locke et al. (2007).*

The total serum proteins were useful in diagnosis of fish diseases *(Mulcathy, 1967).* In the present work, significant decrease in albumin, globulin and total protein.
The protein level differs from species to another and from one period to another after injections.

This is attributed to the *Streptococcus* which causes liver damage that causes decreases of serum protein concentration *(Edvington et al., 1994).* All indicated that infection of fish caused decrease in total protein (28% and 19%).

Also, the globulin level decreased in the 2nd week more than the 1st week decreased till the 4th week. The level of globulin increased in Monosex fish more than in the *Mugil cephalus* and decreased in infected fish than the control one .

Hypoalbuminaemia was also observed in both types of fish after infection with *Streptococcus Kanko* (1989) stated that chronic liver disorder in case of bacterial infection is usually accompanied by hypoalbuminaemia. Both hypogammaglobulinaemia and hypoalbuminaemia confirmed the recorded hypoproteinaemia, which was associated with liver damage (*Maning and Wyatt*, 1984).

The decrease in serum globulin fraction in both acute and chronic exposure to *Streptococcus* and this decrease commonly in the last stages of the experiment, which may be attributed to lymphopenia *(Manning and Wyatt, 1984).* This is due to liver damage where all plasma protein synthesis usually occur in liver except gamma globulins which are produced by lymphocytes *(Khalil, 1998).*

In general the infection with isolated *streptococcus* induced decreasing of total serum protein, hypoalbuminaemia and subsequently hypoglobulinaemia.
The antibody titer level increased from 1st to 4th week. The higher level of antibody titer observed in the control bacteria group and control saline group. And the lowest level observed in the ammonium choloride, then lime and low oxygen treated group.

The groups treated with S. pnumoniae showed lower level of antibody titer, followed by S. iniae and S. fecalis.

This results attributed to the degenerative changes of internal organs resulted from Streptococcus infection that causes lowering the immunity of the fish and so the antibody titer *(Buchanan et al. 2007).*

The Mortality level, Relative level of protection (R.L.P) : The higher mortality level observed in control bacteria group followed by control saline group, while the lowest mortality level observed in ammonium chloride treated group, followed by low oxygen treated group and lime treated group, respectively. Also, the mortality level observed in groups treated with S. pnumoniae of higher mortality level, followed by S. iniae and the least level observed in the groups treated with S. fecalis.

The histological results due Streptococci infection appeared in the form of variable amounts of purulent exudate and/or blood. A yellowish exudate often covered the peritoneum and the epicardium, and was also found in the cranial cavity. In addition, haemorrhages were also observed in muscles, spleen, liver and kidney. The liver showed vacuolar degeneration and necrosis of most hepatic cells and pancreatic acinar cellsm, liver showed wide spread vacuolar degeneration., the spleen showed focal lymphoid depletion with hemolyses of erythrocytes, also the melanomacrophages were degenerated and necrotic. This results greed with those of *Miller and Neely (2004),* where they reported degenerative changes in the liver, spleen, brain, Gills and intestinal cells, due to infection of the fish with Streptococcus infection.

This study concluded that *Streptococcus* diseases are one of the most important bacterial diseases among cultured fish Spps. in Egypt especially *O.niloticus* Since, the results of this work revealed successfully isolation of *Streptococcus* Sps. from infected fish (3 isolates).

Streptococcal infection have immunosuppressive effect and highly pathogenic especially under stress conditions.

The results of economic losses due to enterococci infection indicated that, the higher economic losses observed in the groups of O. niloticus infected with S. pnumoniae control bacteria group, S. faecalis low O2 group and S. pnumoniae lime treate group.

This results agreed with those of *Eyngor et al. (2008) and Locke et al. (2007),* where they reported the S. Pnumonie, iniae and S. fecalis causes severe economic losses through increasing mortality level and percentage in cultured fresh water fish and its hazards effect increased uring stress conditions.

Our results concluded that, stress conditions causes facilitation of the bacteria especially enterococci bacterial group to infection of O. niloticus cultured fish associated with high mortality, weight losses, severe histological changes and severe economic losses to cultured O. niloticus fish farms.

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