Vet.Med.J.,Giza. Vol.46, No.3. (1998): 259-266.

RELATION BETWEEN BACTERIAL LOAD AND HISTAMINE LEVEL IN FISH AND THEIR PUBLIC HEALTH SIGNIFICANCE.

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Received : 24/1/1998
Accepted : 22/3/1998

SUMMARY

Three hundreds and twenty fish samples from Tilapia nilotica and Clarias lazera as fresh water fish and Pagrus sp. and Solea sp. as marine water fishes were collected from Ismailia fish markets as 20 sample from each species/season.

Count, isolation and identification of Enterobacteriaceae organisms as well histamine level were determined in the examined samples by means of thin layer chromatographic methods..

Lowest levels of histamine were recorded from Pagrus sp., Solea sp., Tilapia nilotica then Claroas lazera..

Average counts of AEnterobacteriaceae organisms isolated from muscles of Pagrus sp., Solea sp., Tilapia nilotica then Claroas lazera. caught during autumn, winter, spring and summer were $<10^2$, $<10^2$, $2x10^3$, $4x10^3$, $<10^2$,$<10^2$ $x10^2$ $5x10^2$, 10^3 $<10^2$, $<10^2$, $5x10^3$, 10^4 $2x10^2$, $2x10^3$, 10^4 and 10^4 respectively.

Higher counts of Enterobacteriaceae were recorded during summer due to higher temperatures. Public health importance of histamine and isolated organisms were discussed.

INTRODUCTION

Fish are one of the most important food stuffs used for human consumption . From a fish diet one can obtain not only animal protein , but also the abundant calorific value of fat especially from fatty fish, beside a considerable amount of fat-soluble vitamins (A,D and E) combined phosphorus as well as trace elements, (Jacquot, 1961), therefore it is considered as an excellent medium for the growth of many microorganisms including Enterobacteriaceae.

Histamine as a toxin and the exposure of human

beings to excessive levels represents a health problem.

The detection of histamine in food has great interest from both the hygienic and toxicological viewpoint (Gilbert et al., 1980; Asker et al., 1986 and Vidal-Carow et al., 1990).

Histamine is a pharmacologically important component which is associated with the development of allergic reactions including some forms of food poisoning.

The muscular tissues of fish normally contain large amounts of free histidine which can be decarboxylated to histamine by certain bacteria, these bacteria mainly belong to the mesophilic members of Enterobacteriaceae (Mossel, 1977; Taylor et al., 1978; Yoshinaga and Frank, 1982 and I.C.M.S.F., 1988a).

MATERIALS AND METHODS

320 fish samples from Tilapia nilotica then Claroas lazera. as fresh water fishes and Pagrus sp. and Solea sp. as fishes were collected randomly from Ismailia fish markets. The samples were subjected to the following examination:

I- Estimation of histamine level:

The techniques recommended by A.O.A.C, 1975; Schutz et al. 1976. Voiget et al., 1977 and Hui and Taylor, 1983 were applied.

II- Count of the Enterobacteriaceae microorganisms:

The technique applied was the drop plate metho recommended by I.C.M.S.F., 1978a by usin violet red bile glucose agar.

III- Isolation and identification of th Enterobacteriaceae microorganisms:

The obtained isolates were identifie morphologically (Jensen's modification, cite after Cruickshank et al., 1975), biochemic (Edwards and Ewing, 1972 and Cruickshank a al., 1975).

The biochemically identified isolates whic showed indefinite results were subjected t reidentification by using Entero-tube II-fo confirmation.

RESULTS AND DISCUSSION

From the results achieved in tables (1 & 2) could be concluded that the level of histamine fish muscle was corresponding to th Enterobacteriaceae count and this agrees wi (Sakabe, 1973a; Corlett et al., 1978; Taylor et a 1979; Niven et al., 1981; Reilly and Sntos, 198 and Rohani et al., 1985) and also the level histamine and Enterobacteriaceae count wer higher in Claroas lazera.Tilapia nilotica , Sole sp., then Pagrus sp. This may be attributed to th water environment which received highly pollute water representing to main source of pollution Such waste water is a potential source for th

Table (1): Histamine levels estimated in mg% in examined fish samples allover the year:

Samples	Histamine content mg/100 gram sample											
	Autumn			Winter			Spring			Summer		
	Min.	Max	Aver.	Min.	Max	Aver.	Min	Max.	Aver.	Min.	Max	Aver.
Pagrus sp	0	0	0	0	0	0	0	0	0	14	17	15.05
Solea sp.	0	0	0	0	0	0	0	0	0	17	24	20. 5
Tilapia nilotica	11	14	12.5	6	7	6.5	13	19	16	17	26	21.5
Clarias lazera	13	16	14.5	7	9	8	16	21	19	20	27	23.5

Table (2) : Summarized results of enterobacteriaceae counts in examined fish samples :

Fish species	Season	Enterobacteriacea counts		
		Minimum	Maximum	Average
Pagrus sp. *(sebastes marinus)*	Autumn	0	0	0
	Winter	0	0	0
	Spring	0	0	0
	Summer	1×10^2	1×10^3	2×10^2
Solea sp.	Autumn	0	0	0
	Winter	0	0	0
	Spring	0	0	0
	Summer	1×10^3	3×10^3	2×10^3
Tilapia nilotica	Autumn	1×10^3	3×10^3	2×10^3
	Winter	1×10^2	1×10^3	5×10^2
	Spring	1×10^3	1×10^4	5×10^3
	Summer	2×10^3	2×10^4	1×10^4
Clarias lazera	Autumn	2×10^3	6×10^3	4×10^3
	Winter	3×10^2	2×10^3	1×10^3
	Spring	3×10^3	2×10^4	1×10^4
	Summer	3×10^3	3×10^4	1×10^4

No.=20

Table (3) : Incidence of isolated Enterobacteriaceae organisms from pagrus sp. and solea sp. in every season of the year

Isolates	Pagrus sp.								Solea sp.							
	Autumn		Winter		Spring		Summer		Autumn		Winter		Spring		Summer	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Enterobacter cloacae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
Enterobacter aerogenes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
Proteus mirabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
Escherichi coli	0	0	0	0	0	0	2	10	0	0	0	0	0	0	3	15
Citrobacter freundii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
Proteus morganii	0	0	0	0	0	0	4	20	0	0	0	0	0	0	5	25
Proteus vulgaris	0	0	0	0	0	0	2	10	0	0	0	0	0	0	3	15
Providencia rettgeri	0	0	0	0	0	0	1	5	0	0	0	0	0	0	2	10

No. = Number of the positive samples to the microorganism.

Table (4) : Incidence of isolated Enterobacteriaceae organisms from *Tilapia nilotica* and *Clarias lazera* in every season of the year

Isolates	*Clarias lazera*								*Tilapia nilotica*							
	Autumn		Winter		Spring		Summer		Autumn		Winter		Spring		Summer	
	N0	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Enterobacter cloacae	3	15	1	5	5	25	8	40	2	10	0	0	3	15	5	25
Enterobacter aerogenes	1	5	0	0	1	5	2	10	0	0	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0	1	5	0	0	0	0	0	0	0	0
Proteus morganii	0	0	0	0	1	5	1	5	0	0	0	0	0	0	0	0
Proteus mirabilis	1	5	1	5	5	25	7	35	1	5	0	0	3	15	5	25
Escherichi coli	2	10	1	5	3	15	5	25	2	10	0	0	3	15	5	25
Citrobacter freundii	1	5	0	0	2	10	5	25	1	5	0	0	2	10	3	15
Providencia rettgeri	1	5	0	0	2	10	5	25	0	0	0	0	2	10	3	15
Providencia alcalifaciens	1	5	0	0	3	15	3	15	1	5	0	0	2	10	2	10
Providencia stuartii	2	10	0	0	3	15	3	15	1	5	0	0	2	10	2	10

No = Number of the positive samples to the microorganism.

members of Enterobacteriaceae (Badran et al., 1994). The results were also higher in fresh water fishes (Claroas lazera. & Tilapia nilotica) than in marine fishes (Solea sp. & Pagrus sp.). This may be due to high organic load in fresh water which is favourable for the survival of many genera of bacteria. On the other hand the salinity of marine water inhibits the growth of such bacteria (Robert, 1978). The results were higher in non scally fish (Claroas lazera. & Solea sp.) than scally fish Tilapia nilotica & Pagrus sp.) respectively. This may be due to the absence of scales giving a wide chance to the contaminants to penetrate easily the fish muscles (Baulenger, 1907). The results were higher during summer season when compared with other seasons; this can be attributed to the seasonal variations of the temperature (Goda et al., 1980).

The histamine is produced by certain bacteria that possess the enzyme histidine decarboxylase, which converts histidine to histamine, many bacteria possess histidine decarboxylase but only a few have the capacity for producing large quantities of histamine in short period of time in fish.

Histamine- forming bacteria in fish can be attributed to the differences in fish species, handling procedures and temperature (Kimata, 1961).

The results in tables (3&4) showed that the percentage of isolation of different members of Enterobacteriaceae microorganisms were higher in (Claroas lazera. Tilapia nilotica, Solea sp. then Pagrus sp. The presence of Enterobacteriaceae organisms in the muscles may be attributed to the normal flora of skin, gills and fish intestines (Lerke et al., 1978), if these microorganisms held at favourable growth temperature for sveral hours they grow very rapidly with attendant increase in histamine in the fish muscles (Niven et al., 1981). In this study, the microorganisms responsible for histamine accumulation in fish nearly all belong to family Enterobacteriaceae. This result agree with the findings of Niven et al., (1981); Taylor and Woychik, (1982); Yoshinaga and Frank, (1982) and Okuzumi et al., (1984a).

The Enterobacteriaceae micoorganisms were indicator organisms of public health significance (Mossel et al., 1962). The isolate E.soli can cause gastrointestinal disease (Delopine, 1903), Peritonitis, meningitis, cystitis, pyelonephritis, appendicitis and otitis (Pyatkin and Krivoshein, 1980) and travellers diarrhoea (Mehlman and Romero, 1982); also proteus could be isolated, this organism causes gastroenteritis (Wassef, 1969), urinary tract infections (I.C.M.S.F., 1978b) and food poisoning (Frazier, 1967 and Halstead, 1967). Isolated citrobacter may also cause intestinal and urinary and other pyogenic infections (Krieg and Holt, 1984) and gastroenteritis (Bailly and Scott, 1974). Providencia could be also isolated this may cause human diarrhoea (Cruickshank et al., 1970) and urinary tract infrections (Bailey and Scott, 1974).

Histamine toxicity was done within 10 minutes to 2 hours, there was peppery taste in mouth, and flushing of the facial and neck with feeling of heat, severe headache and cardiac palpation, dizziness, faintness, itching, abdominal cramping

in severe cases, hypotension (Gilbert et al., 1980), allergic reaction (Amonymous, 1980); Frank et al., 1981 and James, 1984).

The Egypt Standards, 1989, 1990a,b, recommended histamine level not more than 10 mg.

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