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REPROTOXIC EFFECT OF AMMONIA ON THE NUTRITION AND GROWTH OF THE FRESHWATER LOACH *LEPIDOCEPHALUS THERMALIS*

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ABSTRACT

The exposure of *L. thermalis* to sublethal concentrations of ammonia for 60 days impaired feed intake, and there was a significant decrease in conversion efficiency, specific growth rate (SGR), and daily growth rate (DGR). The liver and gonadal weight also decreased considerably, as indicated by reduced hepatosomatic index (HSI) and gonadosomatic index (GSI). Gonad maturation in *L. thermalis* was also impaired at sublethal experimental concentrations of ammonium chloride and ammonium sulphate. Moreover, chronic ammonia exposure induced several nuclear pathological conditions in primary oocytes, destruction of ovarian stroma, and reduction in the number of previtellogenic and vitellogenic oocytes, and degeneration of some of the vitellogenic oocytes in *L. thermalis.* The testes of ammonia-exposed fish also showed remarkable pathological changes in spermatocytes and spermatids, and also a reduction in the number of spermatids and spermatozoa. Furthermore, the present study strongly supports the concept that the level and type of dietary components can greatly alter the biologic response of an animal under toxicant exposure.

Keywords: *Lepidocephalus thermalis*, **reprotoxicology, fecundity, ammonia, growth.**

1.INTRODUCTION

Ammonia toxicity is one of the common causes of the death during fish and shell-fish culture. In intensive culture systems the problem of ammonia accumulation needs a careful monitoring and control (Campbell, 1973). The toxicity of ammonia may be a limiting factor in fish farm design and management (Sousa *et al.*, 1974; Hampson, 1976; Barimo and Walsh, 2006; Gena *et al.*,2009; (Ching *et a*l.,2009; Chew *et al.*,2009; Chew *et al.*,2010; Braun *et al.*,2009; Barimo *et al.*,2007)), since this is the main nitrogenous excretory product of fish (Smith, 1972; Campbell, 1973). To be precise, ammonia is highly toxic to aquatic organism and is listed as a regulated toxic pollutant in effluents (Rue and Fava, 1981). Ammonia nitrogen and nitrate nitrogen values are considerably higher in raw sewage effluent. The raw sewage has a range of ammonia nitrogen of 21.7 to 31.5 mg/l and nitrate nitrogen of 2.32 to 6.1 mg/l (Ali, 1992). On the other hand, the values of ammonia and nitrate nitrogen in the fresh water are very low (0.18-0.4 and 0.1-1.0 mg/l respectively).

Ammonia is a chemical irritant, which in its unionized form, is primarily responsible for toxicity (Lloyd and Herbert, 1960). A direct contact with acute concentrations of ammonia causes marked deterioration in organs such as skin, gills and intestine (Eller, 1975). In aquatic environment higher concentrations of ammonia is a predisposition to bacterial gill disease (Wedemeyer and Yasutake, 1977). Unionized ammonia is readily soluble in lipid of cell membranes and so easily taken up by fish gills, whereas larger hydrated and charged ionic ammonia cannot readily pass through the charge-lined hydrophobic micropores of the cell membrane (Hampson, 1976).

Thus many studies have been made on the toxicity of ammonia on fishes because of their high economic value (Palanichamy *et al.*, 1985 a, b; Sarkar and Konar, 1985 a, b; Sarkar and Pramanik, 1987; Neeraja *et al.*, 1987; Sarkar, 1991 a, b; Das and Jana, 2000; Barimo and Walsh, 2006; Gena *et al.*,2009; (Ching *et a*l.,2009; Chew *et al.*,2009; Chew *et*

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al.,2010; Braun *et al*.,2009; Barimo *et al*.,2007). So, for a fish culturist, ammonia is of great concern, which needs careful monitoring and treatment in high density culture systems. Information available in the literature reveal that accumulation of sufficient levels of free ammonia in intensive culture systems is inevitable and may result in lethargy, reduced appetite and food intake, reduced dietary efficiency and growth, more disease susceptibility, morbidity and finally death (Klontz *et al.*, 1985). High levels of ammonia reduce the reproductive potential of different species of fish (Sarkar, 1991 a; Sarkar and Konar, 1985 a, b). The influence of ammonia may retard fertilization membrane formation, which continued to reduce initial cell division.

However, little information is available on the acute and chronic effects of ammonium salts when compared to other toxic pollutants (Kumar and Mukherjee, 1988; Mukherjee *et al.*, 1991), on fish reproduction. So the present investigation was undertaken, principally to evaluate the impact of ammonia intoxication in the gonadal histology of *Lepidocephalus thermalis*, when fed with the five different experimental diets, combined with the measurement of alterations in the physiological traits such as food utilization, feed conversion ratio (FCR), specific growth rate (SGR), daily growth rate (DGR) and feed conversion efficiencies (K_1 and K_2).

2.MATERIALS AND METHODS

Acute toxicity bioassay

Single species toxicity tests were performed in the laboratory according to the recommendations of Sprague (1969) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). The test fishes (*L. thermalis*) were maintained in well water at a temperature of 29 $\pm 1^0$C, pH 7.1±0.2 and dissolved oxygen 6.76±0.5 ml /l. The fish were not fed during 96 hr static toxicity tests. The bioassay was performed in two phases:

1.Exploratory tests
2.Final tests from which estimation of median lethalconcentrations (LC_{50}) are determined.

The test solutions were made by dissolving requisite amount of analar grade ammonium chloride (NH_4Cl) and ammonium sulphate [$(NH_4)_2SO_4$] crystals. A series of exploratory tests were made to determine the approximate range of test concentrations to be used for the final tests. The highest concentration at which all the fish survived for 96 h and the lowest concentration at which most of the fish died in 24 h constituted the two extremes of the test range. The exploratory tests were conducted with five test animals per concentration.

The final tests were conducted in a logarithmic series of concentrations and four replicates of 10 individuals were tested at each concentration. Control groups were reared separately. The photoperiod regime used in the bioassay was normal 14 h L/10 h D cycle. The bioassay water was changed daily by a siphon to minimize stress effects. All concentrations reported in the study were calculated from the levels of ammonia solutions added at the start of the tests. Mortality was recorded daily and the assays were terminated at 96h. The median tolerance limit (LC_{50}) of the ammonium salts were calculated by straight-line graphical interpolation methods and checked with a computer-programmed Probit analysis (Busvine, 1971).

Experimental procedure:

Sexually maturing *L. thermalis* were selected from the laboratory stock, placed on damp paper to toweling to remove excess water, and then weighed (±0.1 mg. accuracy). The day prior to weighing, the fish were not fed. The initial wet weight of the fish was 415.0 ± 5.0 mg. The growth chambers were all 10-l round glass aquariums (28 cm.dia x 15 cm.ht). The acclimation regime and the experimental conditions were identical with those reported earlier. Dissolved oxygen was maintained above 6-7 mg/l and temperature at 29 ± 1^oC. Water was renewed every 24 hrs. The experimental design consisted of exposing 15 test animals to 3 levels each of Ammonium chloride and Ammonium sulphate (0.02, 0.10 and 0.20 mg/l) with three replicates at each level. This was done for all the five different types of feeds separately. Ammonia levels were maintained by dosing with analar grade NH_4 Cl and $(NH_4)_2SO_4$. Dosing with ammonium salts started the next morning and continued for 60 days. The fish in each aquarium were fed at 10% body weight daily. Due to differences in size and appetite, food consumption at the different treatment levels varied at the end of experiment.

Test parameters and statistical analyses:

Responses of fish to Ammonium chloride and Ammonium sulphate exposure were evaluated by studying growth parameters such as food intake, weight gain and conversion efficiencies (K_1 and K_2). At the end of the experiment, the fish were blotted dry and the wet weight of each fish was recorded individually, following which they were killed with a blow on the back of the head. Ovary and liver were dissected out, and weighed. All weights represent the mean of the pooled weight of all fish in each aquarium. The length and weight of ovary, GSI, fecundity, and diameter of mature ova were measured in each fish, to study the reproductive parameters. All data were analyzed statistically by analysis of variance (ANOVA) followed by Post-ANOVA test of comparisons of means by Duncan's multiple range test when a range of means was compared.

Gonad histopathology

To determine the pathological changes in the gonads after 60 days of ammonia treatment, portions of ovaries and entire testes from control and experimental fish were fixed in Bouin's solution. After being fixed for 48 hrs, they were washed in tap water for 3-4 hrs, dehydrated through a graded series of ethyl alcohol, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned serially at a thickness of 6 μm and stained with Harris' haematoxylin and Mason's trichrome. Morphometric measurements of healthy oocytes from each test group were made with calibrated ocular micrometer at X 100 / X 400. The percentage frequency of oocytes in different stages of development along

with the occurrence of atresia was estimated for control and experimental fish.

3.RESULTS

Lethal responses

Ammonia in the form of ammonium chloride and ammonium sulphate is found to be acutely toxic to the freshwater fish *L. thermalis* between 2.0 mg/l and 4.5mg/l. The toxic effects of various concentrations of ammonia as ammonium chloride and ammonium sulphate on mortality are given in Table 1. From the acute lethality data, the median tolerance limits (LC_{50}) for 24, 48, 72 and 96 hrs were calculated by probit analysis as 3.222, 3.148, 2.904 and 2.736 mg/l for ammonium chloride, and 3.566, 3.320, 2.970, and 2.621 mg/l for ammonium sulphate respectively. It has been observed that the resistance of the test individuals to ammonia decreased with increasing experimental duration. Thus toxic response was a function of the concentration of ammonia compounds and duration of exposure. Using toxicological statistics (Busvine, 1971), the toxicity of ammonia to *L. thermalis* was evaluated and the agreement was found to be too good at 5% critical value (Table 2).

Feeding responses :

Food consumption and other feeding parameters of *Lepidocephalus thermalis* fed with five test feeds and exposed to different concentrations of ammonium chloride and ammonium sulphate solutions for 60 days are shown in Table -3. All fish survived the test conditions and their behaviour appeared normal except at ammonia intoxication. Food consumption decreased with increasing concentrations of ammonium chloride and ammonium sulphate for the fish fed all the five test feeds. Fish reared in ammonia-free water consumed 16.4, 15.6, 20.6, 17.3 and 21.4 mg / fish / day for the test feeds I to V respectively.

Pathophysiological responses:

Food conversion ratio (FCR) ranged between 6.09 and 7.73 for the control fish fed on various test feeds. FCR increased in *L. thermalis* with increase in the concentrations of both ammonium chloride and ammonium sulphate for all the test diets. Increase in FCR was significant ($P<0.05$) at 0.20 mg/l (Table 3).

Net conversion efficiencies (K) also decreased for the fish exposed to higher concentrations of ammonium salts (Table - 3). Hepatosomatic index (HSI) decreased with the increasing concentrations of ammonium chloride and ammonium sulphate in *L. thermalis* fed all the five test diets, when compared to the control.

Reprotoxic responses:

The ovary length of *L. thermalis* reared in ammonia - free water fed with the five different types of test feeds for 60 days ranged between 14.6 and 17.2mm. However there was a decrease in the length of ovaries of fish exposed to both ammonium chloride and ammonium sulphate (Table 4). The weight of ovaries of control fish ranged from 67.31 to 114.73 for the test feeds I to V. The fish fed test feed V had the highest weight gain of ovary. The weight of ovaries also decreased in the females exposed to ammonia for a period of 60 days (Table 4). Hence there was a decrease in GSI of ammonia - exposed *L. thermalis* from the normal fish.

Fecundity responses:

The mean absolute fecundity of control fish fed with test feed I was 483, that of feed III was 856, and the mean absolute fecundity was the highest (986) in the fish fed test feed V (Table 4). But there was a corresponding decrease in the absolute fecundity of the fish exposed to both ammonium chloride and ammonium sulphate fed with the different feeds for 60 days. Similarly the relative fecundity also decreased in ammonia-exposed fishes fed test feeds I to V (Table 4).

Responses of oocyte growth and gonadal pathology:

The average diameter of oocytes and their nuclei, and the occurrence of oocytes in the various stages of development for the control and ammonia-exposed *L. thermalis* are shown in Table 5. Since there is no significant difference between the various experimental diets, the data are pooled and the mean values are computed.

The frequency of mature oocytes was significantly reduced ($P<0.05$) and conversely the frequency of occurrence of atretic oocytes was highly significant ($P<0.01$) in the ovaries of ammonia-poisoned fish. Mean oocyte diameter of mature ova was significantly reduced in the female *L. thermalis* exposed to ammonium chloride and ammonium sulphate for 60 days.

In the ovaries of control fish *L. thermalis*, the immature and mature oocytes appeared normal with well-defined oocyte differentiation and vitellogenesis . Histologically detectable alterations in the morphology of oocytes in the various stages of development were observed after 30 days of exposure to 0.2 mg / l of ammonium chloride and ammonium sulphate solutions. Ammonia exposures induced several nuclear pathologies in the primary oocytes such as karyolysis, karyorrhexis, hypo-chromatin nucleus, intra-nuclear bodies and necrosis. Destruction of many of the primary oocytes was detected. After 60 days of ammonia exposure, destruction of ovarian stroma, reduction in the number of pre vitellogenic and vitellogenic oocytes were observed. Toxified previtellogenic oocytes displayed acidophilic intra-nuclear inclusions and necrosis. Long-term ammonia exposure resulted in loosening of oolemma and hydrophilic and vacuolar degeneration of vitellogenic oocytes. Some of the ripe oocytes began to regress and became atretic. Histology of control testis of *L. thermalis* showed large number of spermatocytes, spermatids and spermatozoa. Examination of 0.20mg/l ammonia-treated testis disclosed striking pathological effects such as nuclear pyknosis, cytokaryolysis, and necrosis of spermatocytes and spermatids. The number of spermatids and spermatozoa was greatly reduced.

Table-1. *L.thermalis* : Percentage mortality and median tolerance limits (LC_{50})at 29 ± 1^0 C under laboratory conditions.

Concentration of NH_4Cl (mg/l)	Mortality (%)					Mortality (%)			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	
2.00	-	-	-	-	2.00	-	-	-	20
2.25	-	-	-	10	2.25	-	-	-	30
2.50	-	-	30	40	2.50	-	-	20	40
2.75	-	20	40	50	2.75	-	10	30	50
3.00	30	40	40	60	3.00	20	40	50	60
3.25	50	60	70	80	3.25	20	40	70	80
3.50	60	70	90	100	3.50	30	50	80	90
3.75	70	80	90	100	3.75	60	80	90	100
4.00	80	100	-	-	4.00	80	90	100	-
4.25	90	-	-	-	4.25	100	-	-	-
4.50	100	-	-	-	4.50	100	-	-	-
Lethal	4.500	4.000	4.000	3.750	**Lethal**	4.250	4.000	4.000	3.750
Median lethal	3.322	3.148	2.904	2.736	**Median lethal**	3.566	3.320	2.970	2.621
Sub lethal	0.033	0.031	0.029	0.027	**Sub lethal**	0.035	0.033	0.029	0.026
	Sublethal dose = LC_{50} X 0.01 Application factor.								

Table-2. *L.thermalis* : Summary of computerised (APPLE - IIB) regression analysis (BUSVINE,1971) of median lethal concentrations of ammonium compounds

Toxic chemical	Time hrs	Slope (b value)	Intercept (a value)	95 % Fiducial limit		LC_{50}
				m_1	m_2	mg / l
	24	12.3879	-11.9366	1.4895	1.5532	3.322
Ammonium	48	14.5350	-13.5268	1.4719	1.5241	3.148
chloride	72	11.3212	-11.8834	1.4302	1.4957	2.904
	96	13.5095	-12.2696	1.4100	1.4643	2.736
	24	17.4947	-16.8793	1.5314	1.5729	3.566
Ammonium	48	13.6188	-16.7600	1.4950	1.5472	3.320
sulphate	72	13.4520	-13.1622	1.4457	1.4997	2.970
	96	9.16280	-6.85450	1.3837	1.4532	2.621

Table-3. *L.thermalis* : Influence of different concentrations of ammonium compounds on food conversion ratio (FCR) and net conversion efficiency (K_2) when fed with different feeds for 60 days.

Test feed	Control	Conc. of Ammonium chloride (mg/l)			Conc. of Ammonium sulphate (mg/l)		
		0.02	0.10	0.20	0.02	0.10	0.20
Food conversion ratio (FCR)							
I	6.09	6.42 *a*	14.08 *b*	18.15 *c*	6.49 *a*	15.62 *b*	16.39 *b*
II	6.43	10.06 *a*	13.29 *b*	20.22 *c*	9.01 *a*	11.89 *b*	13.31 *c*
III	6.99	7.85 *a*	12.09 *b*	16.61 *c*	7.34 *a*	8.07 *a*	17.89 *b*
IV	7.73	11.58 *a*	13.04 *a*	20.61 *b*	11.15 *a*	15.75 *b*	19.39 *c*
V	6.6	7.14 *a*	10.45 *b*	20.39 *c*	6.88 *a*	9.67 *b*	17.73 *c*
Net conversion efficiency (K_2)							
I	22.06	21.94	9.7	9.15	24.55	10.06	8.11
II	20.22	14.84	10.77	6.89	15.06	13.46	9.37
III	18.31	18.96	11.59	7.69	20.74	17.93	7.72
IV	16.82	11.99	10.38	6.2	12.81	9	7.15
V	19.74	21.39	14.19	6.48	20.26	14.9	7.89

Different italic alphabets in the same row indicate significant difference ($P<0.05$) between different concentrations for the same feed.

Table-4. ***L.thermalis*** : Influence of chronic ammonium chloride (0.2 mg/l) and ammonium sulphate (0.2 ml/l) poisoning on ovarian parameters fed with different test diets after 60 days.

Test feed	Ovary Length (mm)	Ovary Weight (mg)	GSI (%)	Absolute fecundity	Relative fecundity	Ovum Diameter (mm)
			Control			
I	14.7 ± 0.8	67.31 ± 5.45	11.33 ± 0.81	483 ± 39 *d*	826 ± 45	0.51 ± 0.03
II	15.2 ± 0.6	88.29 ± 6.14	16.84 ± 0.53	672 ± 47 *c*	1286 ± 62	0.51 ± 0.03
III	16.2 ± 1.1	98.34 ± 4.19	16.48 ± 0.65	856 ± 42 *b*	1438 ± 59	0.52 ± 0.02
IV	14.6 ± 0.9	93.56 ± 5.33	17.34 ± 0.42	825 ± 38 *b*	1481 ± 67	0.51 ± 0.03
V	17.2 ± 0.4	114.73 ± 6.15	20.61 ± 0.85	986 ± 65 a	1820 ± 79	0.53 ± 0.02
Ammonium chloride - exposed						
I	12.5 ± 1.2	36.85 ± 4.27	8.96 ± 0.64	197 ± 26 *b*	487 ± 37	0.45 ± 0.03
II	12.3 ± 0.4	41.75 ± 5.28	11.89 ± 0.31	215 ± 24 *b*	604 ± 31	0.44 ± 0.03
III	15.5 ± 0.7	62.81 ± 5.46	14.18 ± 0.37	329 ± 31 *a*	735 ± 43	0.45 ± 0.04
IV	12.7 ± 0.5	47.28 ± 4.06	13.09 ± 0.28	148 ± 20 *c*	418 ± 35	0.44 ± 0.03
V	12.8 ± 0.6	59.33 ± 3.52	16.54 ± 0.59	317 ± 29 a	928 ± 38	0.45 ± 0.03
Ammonium sulphate-exposed						
I	13.4 ± 0.7	28.53 ± 3.17	8.08 ± 0.25	165 ± 28 *c*	481 ± 39	0.44 ± 0.03
II	12.6 ± 0.9	31.28 ± 2.40	7.32 ± 0.19	183 ± 23 *c*	440 ± 29	0.42 ± 0.03
III	12.3 ± 1.1	42.75 ± 4.38	11.73 ± 0.55	249 ± 35 *b*	692 ± 46	0.44 ± 0.02
IV	14.5 ± 0.8	39.41 ± 3.11	9.59 ± 0.21	277 ± 42 *a*	697 ± 51	0.42 ± 0.03
V	15.2 ± 1.2	54.69 ± 5.26	13.31 ± 0.68	314 ± 30 a	773 ± 48	0.43 ± 0.04

Different italic alphabets in the same column indicate significant difference ($P<0.05$) between feeds for control and exposed separately.

Table-5. *L.thermalis* : Oocyte dimensions and percentage frequency of oocytes in different stages in the control and ammonia -exposed (0.2mg/l) fish after 60 days (mean ± SEM).

Parameter	Oocyte Stages					Atretic oocyte
	1	2	3	4	5	
Mean oocyte diameter □□□	16.5 ± 5.6	104.8 ± 21.3	197.2 ± 42.5	369.1 ± 37.4	488.6 ± 21.5	
Mean nucleus diameter □□□	10.7 ± 1.4	61.3 ± 5.9	114.8 ± 5.2	182.5 ± 6.1	-	5.4
Occurrence (%)	9.5	12.7	11.4	26.8	34.2	
Mean oocyte diameter □□□	13.7 ± 3.2	90.5 ± 15.8	172.6 ± 34.1	349.3 ± 29.5	405.2 ± 11.7	
Mean nucleus diameter □□□	10.2 ± 0.8	58.4 ± 3.5	108.3 ± 5.7	171.6 ± 4.2	-	29.6
Occurrence (%)	14.7	19.5	12.6	15.2	8.4	
Mean oocyte diameter □□□	14.3 ± 2.5	87.4 ± 12.6	178.2 ± 26.5	341.9 ± 22.7	412.9 ± 10.3	
Mean nucleus diameter □□□	9.8 ± 1.5	55.7 ± 4.3	106.4 ± 5.1	168.8 ± 4.6	-	25.1
Occurrence (%)	13.5	1.2	14.6	16.9	11.7	

4.DISCUSSION

Ammonia tolerance:

The median tolerance limits of *L. thermalis* at 24, 48, 72 and 96h were calculated as 3.32, 3.15, 2.9 and 2.74 mg/l for ammonium chloride and 3.57, 3.32, 2.97 and 2.62 mg/l for ammonium sulphate solutions respectively. The 96 h LC_{50} value of un-ionized ammonia to channel catfish *Ictalurus punctatus* at 30^{o}C was 3.8 mg/l (Colt and Tchobanoglous, 1976). In a study conducted on five species of British fish, Ball (1967) found that the incipient LC_{50} values for all fish species were essentially equal to 0.49 mg/l. Ball (1967) found that rainbow trout (*Salmo gairdneri*) were more sensitive to un-ionized ammonia than bream (*Abramis brama*), perch (*Perca fluviatilis*), roach (*Rutilus rutilus*) and rudd (*Scardinus erythrophthalmus*). Based on short-term lethal toxicity tests, Wuhrmann and Woker (1948) reported that only un-ionized ammonia was toxic to fish and that ionized ammonia had little or no toxicity. Rice and Strokes (1975) found that the tolerence of fry of rainbow trout to un-ionized ammonia was slightly less than the adults. The LC_{50} value of *Sarotherodon mossambicus* tested at different concentrations of di-ammonium phospate was 0.55 g/l at 96 h (Palanichamy *et al*., 1985 a). The 96 h LC_{50} value determined for *Ictalurus punctatus* was 1600 μg/l NH_3-N (Colt and Tchobanoglous, 1978).

Feeding and growth responses:

The decreased food consumption at higher concentration of ammonia may be due to loss of appetite (Palanichamy *et al.,* 1985 a). Moreover, the ammonia-exposed fish became stressed and hence remained lethargic, lost appetite and anorexic (Rice and Strokes, 1975; Barimo and Walsh, 2006; Gena *et al*.,2009; (Ching *et a*l.,2009; Chew *et al*.,2009; Chew *et al*.,2010; Braun *et al*.,2009; Barimo *et al*.,2007). This had resulted in lower consumption of feed. According to Lubinski *et al.,* (1978 a, b), a low concentration of ammonia stimulated the general locomotor activity of blue gills while higher levels depressed the activity. That might be the reason for the almost normal food consumption in *L. thermalis* at lower (0.02 mg/l) concentration and very low food consumption at higher (0.2 mg/l) concentration of ammonia, irrespective of the type of test feed. A similar impairment of feeding was noticed in *Ictalurus punctatus* when subjected to short-term toxicity of nitrogenous compounds (Colt and Tchobanoglous, 1976). The average feeding rate of test fish, *Barbus stigma* decreased significantly in different concentrations of endosulfan (Manoharan and Subbiah, 1982). Woltering *et al* (1978) also reported a decrease in the predatory activity of largemouth bass *Micropterus salmoides* exposed to 0.63 and 0.86 mg/l of ammonia concentration.

Palanichamy *et al*., (1985 a) observed that growth of *Sarotherodon mossambicus* declined with increased concentration of ammonia in the medium. There was a reduction in growth of channel catfish *Ictalurus punctatus* when reared at sublethal concentration of ammonia (Colt and Tchobanoglous, 1978). Growth - limiting influence of ammonia was observed in a number of fish like rainbow trout (Forster and Smart, 1976), *Soles solea* and *Scophthalmus maximus* (Alderson, 1979), *Anguilla anguilla* (Sadler, 1981), and *Ictalurus punctatus* (Robinette, 1976). Specific growth rate also decreased in *Barbus stigma* exposed to different sublethal concentrations of endosulfan (Manoharan and Subbiah, 1982). Net conversion efficiency decreased in *S. mossambicus* exposed to increasing concentrations of diammonium phosphate (Palanichamy *et al.,* 1985 a), and in *Barbus stigma* exposed to endosulfan (Manoharan and Subbiah, 1982).

At sublethal levels, ammonia may reduce growth by several physiological mechanisms - imposition of additional energy demand caused by the switching over to alternate detoxification pathways (Olson and Fromm, 1971), inhibition of uptake of sodium ions (Maetz, 1972), increased loss of ions by more urine flow (Lloyd and Orr, 1969), switching over to urea excretion at high concentration of ammonia in the medium (Groves and Kogel, 1973), reduction of appetite (Kawamoto, 1961), reduction of oxygen uptake ability due to gill damage (Mayer and Kramer, 1973; Kevin and Nora, 2009; Mitchell and Lambertii 2005; Ian et al.,2000; McKenzie et al.,2008; James and Ann 2006; Kendra 2009; Gennadi et al.,2008; Wicks et al.,2002), and damage to various internal tissues and organs (Larmoyeux and Piper, 1973). Since damage to the gills or other organs may be irreversible, their effect would tend to be cumulative. Therefore, over a long period of time, ammonia may have a greater detrimental effect on somatic growth. The decreased HSI in *L. thermalis* fed with all the test diets under exposed condition indicates the increased rate of metabolic activity and the effort taken by the fish to thrive with the stress, by utilizing the hepatic reserves. This observation was supported by the results of Sastry and Agarwal (1976) in *Heteropneustes fossilis* exposed to sublethal carbon tetrachloride. Belliyappa and Reddy (1986) also observed an elevated metabolic rate of the *H. fossilis* resulting in more utilization of food energy for the purpose of maintenance.

Ovarian growth and fecundity responses:

Little information was available about the effect of ammonia on the cytomorphological characteristics of ovaries, but the effects of other pollutants were reported. Phenol or sulphide could reduce the GSI in sexually maturing female carp *Cyprinus carpio* when exposed for 30 days, suggesting the inhibitory effect of these pollutants on the development and maturation of the ovary (Kumar and Mukherjee, 1988). Organophosphorous pesticides impaired the ovarian maturation in the fresh water teleost *Heteropneustes fossilis* (Singh and Singh, 1980). Exposure of sexually mature *Cyprinus carpio* to sublethal concentrations of phenol and sulphide for 45 days reduced GSI significantly, and the major event that took place was the gradual enlargement of the ovary with concomitant formation of yolky oocytes (Mukherjee *et al*., 1991). In *L. thermalis* there was a significant decrease in GSI and also reduced fecundity indicating the impairment of ovarian development and maturation. According to Sarkar (1988), any decrease in the fecundity and maturity index of fish is due to the accumulation of toxic products like ammonia in water.
Chronic exposure of the fathead minnows, *Pimephales promelas* to mercuric chloride resulted in significant reproductive impairment, no spawning in higher

concentrations and no females were mature at the highest concentration, but all control females spawned (Snarski and Olson, 1982). Phenol and sulfide at very low concentrations can impair gonadal maturation and steroidogenesis (Mukherjee *et al.*, 1991; Kevin and Nora, 2009; Mitchell and Lambertii 2005; Ian et al.,2000; McKenzie et al.,2008; James and Ann 2006; Kendra 2009; Gennadi et al.,2008; Wicks et al.,2002). Similarly continuous exposure to 28% refinery effluent caused severe sublethal damage to flagfish, *Jordanella floridae* and caused a delayed and significantly less frequent spawning with fewer eggs per spawn, compared to controls (Rowe *et al.,* 1983). Since ammonia exhibits a wide variety of biological influence on fish life (Sarkar and Konar, 1985b; Sarkar and Pramanik, 1987), it is reasonable to predict that excess of ammonia in the medium results in the reduction of the reproductive potential of fish, as high levels of ammonia are known to decrease reproduction in fish (Sarkar and Konar, 1985a,b). Kapur *et al.,* (1978) observed that the pesticide fenitrothion reduced the ovarian activity of *Cyprinus carpio*. The same effect was noticed in *Salvelinus fontinalis* (Freeman and Idler, 1975) when treated with polychlorinated biphenyl.

GSI of fish fed test feed -V without ammonia exposure in the present study was the highest ($P<0.05$). In the same manner, the experimental fish *L. thermalis* exposed to sublethal concentration (0.2 mg/l) of ammonium chloride and sulphate fed test feed-V also showed a significantly higher value of GSI. This might be due to the peculiar combination of ingredients in the test feed-V containing 40% of the powdered alga *S. maxima*. Since *L. thermalis* is a herbivore, feeding on the small algae and detritus at the bottom of the pond, it could thrive well even in a medium containing toxic substances like ammonium compounds with augmented gonad development and maturation resulting in a higher ovary weight, GSI and fecundity, compared to the exposed fish fed all other experimental feeds.

A similar efficient feed which produced next higher ovary weight, GSI and fecundity in the exposed fish *L. thermalis* was test feed-III in that the efficiency was attributed to the type of source of protein and the increased amount of carbohydrate in the diet. Test feed-III was marked by 60% of groundnut oil cake as the source of protein and 19.5% of raw rice as the source of carbohydrate.

Pathologic responses of gonads:

The cytomorphological state of the follicles in the ovary coincides with the physiological state of the animal (Horvath, 1985). Few observations are available on the effects of ammonium compounds on the histopathology of gonads in fishes. Chronic toxicity of mercuric chloride caused significant reproductive impairment that no spawning occurred in the fathead minnow *Pimephales promelas* (Snarski and Olson, 1982), because of degeneration of maturing oocytes. The pathological state of nuclei and subsequent destruction of most of the primary and vitellogenic oocytes were distinct in *Pimephales promelas*. Rowe *et al.,* (1983) also observed destruction of developing and maturing oocytes at various stages of maturation in *Jordanella floridae*. Hose *et al.,* (1981) reported the histological retardation of egg development in flatfish by certain polycyclic aromatic hydrocarbons. Singh and Singh (1981) observed that the organochlorine compounds reduced the gonadotrophic potency in the ovary of the freshwater catfish *Heteropneustes fossilis*. A decrease in the size of vitellogenic oocytes due to decreased or inhibition of vitellogenesis and ovarian import of lipids and egg yolk proteins from liver was remarkable in *Cyprinus carpio* exposed to phenol and sulphide (Kumar and Mukherjee, 1988; Mukherjee *et al*., 1991). The brook and brown trout also showed histopathological changes in the ovary and consequent reduction in the reproductive capability, under the influence of the insecticide DDT (Burdick *et al.,* 1972). These observations in different teleosts support the microscopic diagnosis of histomorphological dystrophy and necrosis in the ovaries of *L. thermalis*.

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