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IONIC REGULATION OF THE BLOOD IN THE CAT FISH CLARIAS LAZERA UNDER THE EFFECT OF EXPERIMENTAL ACID POLLUTION

By

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ABSTRACT

The acute and chronic treated fish showed significant decrease in both Na^+ and Cl^- levels. K^+ levels showed a higher values in the acute case, but an adaptation appears to take place in the chronic case. An increased in the concentrations of Ca^{2+} was observed during both acute and chronic acid exposures.

Na^+, K^+ and Ca^{2+} ions efflux showed a significant increase 24 hrs following the transfere of fish to pH 3.1. A rapid decrease of serum osmolality occured at both pH levels (5.4 and 4.7), after chronic acid exposure. This decrease was observed throughout the whole of the experimental period. (12 weeks).

INTRODUCTION

Electrolytes are distributed in solution throughout all the body fluids. They have various functions, the most important of which are to contribute a majority of the osmotically active particles, to provide buffer systems and mechanisms for the regulation of pH (acid - base balance), and to give the proper ionic balance for normal neuro - muscular irritability and tissue function (West, 1957). Disturbances in acid - base balance osmoregulation have been emphasized as key toxic mechanisms leading to fish death (Packer., 1979; Graham and Wood, 1981; Mc Donald and Wood, 1981; Booth et al, 1982 and Mc Donald, 1983 b).

The goal of the present study was to investigate the physiological effects of acute and chronic acid stress on the fresh water fish Clarias lazera. Serum Na^+, K^+, Ca^{2+} and Cl^- were measured as indices of ionioregulatory disturbances.

MATERIALS AND METHODS

Specimens utilized in the present investigation are freshwater fish Clarias lazera, ranging in length from 250 to 300 mm and weighing from 150 to 200 g in weight. They were collected during the summer months June, July and August of the year 1989 and 1990, from Lake Maruit near Alexandria, Egypt. All animals were acclimated for at least two weeks under controlled laboratory conditions. They were fed on commercial dry pellets adlibitum.

Exposure to acid as pure sulphuric acid (H_2SO_4), was carried out using static renewal technique in which test water was periodically replaced, usually once every 24 hr, by a new fresh one of the same composition. Acid concentration to which the fish were subjected was pH 3.1, TL_m pH level for 96 hr exposure and chronic (pH 5.4 and 4.7) for 12 weeks of exposure, in comparison with a control group at pH 7.1.

Blood was sampled for electrolytes assays (by severing the caudal peduncle), allowed to clot and then centrifuged at 8000 for 5 minutes, and serum was stored under refrigeration until the time of analysis.

Serum Na and K were determined by the quantitative analysis of Na and K in serum using ion selective electrode measuring method (Proda and Simon, 1970).

Serum calcium was quantitatively determined using the Cresolphalein complex (CPC) calorimetric method.

Colorimetric determination of chloride ions in serum was undertaken with the TPTZ - mescury complex method (Feld Kamp, 1974).

Determination of the osmotic activity (osmolality) of serum was determined by using the Beckman ASTRA Automated State-Routine Analyzer systems after chronic acid exposure. Comparison of the experimental results with controols was made by student's t-test.

RESULTS

I- In serum samples:

Serum Na concentration was significantly decreased from the control value (105 $\pm$ 2.5 mmol / L) to 94.66 $\pm$ 0.33 mmol / 1 ($p < 0.025$) after 24 hours after of exposure to pH 3.1.

The 72 hours exposure showed a nonsignificant decrease, which become significantly lower than the control value at the end of the experiment 93,33 $\pm$ 2.02 mmol / L ($p < 0.025$). In contrast serum potassium content exhibited a remarkable increase (4.13 $\pm$ 0.03 mmol / L) as compared to their corresponding controls (2.26 $\pm$ 0.08 mmol / L) after 24 hours exposure which remained significantly higher till the 96 hours period (Table 1)

Chronic exposure of pH 5.4 and 4.7 for 12 weeks caused major changes in serum Na^+ content (Table 2), a marked decrease from the control level (117.0 $\pm$ 0.81 mmol / L) was detected starting from the first week of treatment. pH 5.4 caused a drop of the serum Na level to 106.66 $\pm$ 1.453 mmol / L ($p < 0.025$) after the 12 weeks of exposure. The effect of lower pH 4.7 was more severe, since the drop of the serum Na^+ level after 12 week of exposure was highly significant reaching the value of 96.33 $\pm$ 1.453 mmol / L ($p < 0.001$).

Results for serum K concentrations of fish exposed chronically to pH 5.4 and 4.7 respectively are compared in Table (3) with that of controls. A gradual recovery to the control level was observed in the 11 and 12 week of exposure to pH 5.4 reaching value of 2.75 $\pm$ 0.02 and 2.48 $\pm$ 0.04 mmol / L comparable to the control values (2.2 $\pm$ 0.20 and 240 $\pm$ 0.12 mmol / L) respectively.

With respect to the pH 4.7 treated group, significant elevations from the7 control values was observed for 11 weeks of exposure. In the 12 week, only the value obtained was not significant from the control value.

Serum Ca^{2+} concentration of the acute acid treated fish showed a transient decrease from the control level (11.40 $\pm$ 0.15 mg / dl) to 10.16 $\pm$ 0.12 mg / dl after 24 hours of exposure followed by a significant increase at the 96 hr of exposure (11.9 $\pm$ 0.05 mg / dl) $p < 0.05$.

Serum Cl concentration showed a progressive highly significant decrease during the whole experimental period ($p < 0.001$) reaching a level of 57.08 $\pm$ 0.61 mmol / 1 by the 96 hr exposure from the control level of 135.33 $\pm$ 0.88 mmol/L. (Table 4).

Table (1): Changes in serum sodium and postassium concentrations juvenile <u>Clarias lazera</u> during exposure to acidified water (pH 3.1) over a 96-h period.

	Exposure period (hours)	Range	Mean ± S.E.	S.D.	% Coefficient of variation	Level of significance
Sodium (Na^+) (mmol/l)	Control	100-108	105.000±2.517	4.359	4.151	
	24 h	94- 95	94.667±0.333	0.577	0.609	P < 0.025
	48 h	87- 90	88.000±1.000	1.732	1.968	P < 0.005
	72 h	99-102	100.333±0.882	1.527	1.522	N.S
	96 h	90- 97	93.333±2.027	3.512	3.763	P < 0.025
Potassium(K^+) (mmol/l)	Control	2.1-2.4	2.267±0.088	0.153	6.739	
	24 h	4.0-4.3	4.133±0.088	0.153	3.696	P < 0.001
	48 h	2.9-3.1	3.000±0.058	0.100	3.333	P < 0.005
	72 h	2.5-2.9	2.700±0.115	0.200	7.407	P < 0.05
	96 h	3.4-3.8	3.633±0.120	0.208	5.729	P < 0.001

- Each mean result corresponds to a mean value of the three experiments.

S.E. = Standard error.

S.D. = Standard deviation.

Table (2) Changes in serum sodium concentration (expressed as mmol/l) of juvenile Clarias lazera exposed to different treatment with H_2SO_4, pHs 7.1 (control), 5.4 and 4.7 over a 12-week period.

Exposure period (weeks)	Range	Mean±S.E.	S.D.	%Coefficient of variation	Level of Significance
First week	116-118	117.00±0.816	1.414	1.209	
	100.5-104	102.16±1.014	1.756	1.719	P<0.001
	91-95	93.00±1.155	2.000	2.150	P<0.001
Second week	112.5-116	114.25±1.429	2.475	2.167	
	91.0-94	92.33±0.882	1.527	1.654	P<0.001
	70.0-78	74.66±2.404	4.163	5.576	P<0.001
Third week	115-117	116.00±0.816	1.414	1.219	
	106-110	107.33±1.333	2.309	2.152	P<0.01
	113-120	117.00±2.082	3.605	3.082	N.S.
Fourth week	113-117	115.00±1.633	2.828	2.459	
	97-98	97.33±0.333	0.577	0.593	P<0.001
	89-93	91.00±1.155	2.000	2.198	P<0.001
Fifth week	113-118	115.50±2.041	3.535	3.061	
	91-95	92.66±1.202	2.082	2.246	P<0.001
	80-87	82.66±2.186	3.786	4.579	P<0.001
Sixth week	114-120	117.00±2.449	4.243	3.626	
	91-94	93.00±1.000	1.732	1.862	P<0.001
	89-94	91.33±1.453	2.517	2.755	P<0.001
Seventh week	115-115	115.00±0.000	0.000	0.000	
	99-103	100.66±1.202	2.082	2.068	P<0.001
	113-117.5	114.83±1.364	2.363	2.058	N.S.
Eighth week	117-118	117.50±0.408	0.707	0.602	
	113-117	115.00±1.155	2.000	1.739	N.S.
	115-116	115.66±0.333	0.577	0.499	P<0.05
Ninth week	116-118	117.00±0.816	1.414	1.209	
	96-97	96.66±0.333	0.577	0.597	P<0.001
	100-109	103.33±2.848	4.933	4.774	P<0.01
Tenth week	112-114	113.25±0.612	1.061	0.936	
	96-98.5	97.33±0.726	1.258	1.293	P<0.001
	100-107	103.00±2.082	3.605	3.500	P<0.01
Eleventh week	113.5-115	114.25±0.612	1.061	0.928	
	101-103	102.16±0.601	1.041	1.019	P<0.001
	109.5-122	110.50±0.764	1.323	1.197	P<0.025
Twelfth week	112-114	113.00±0.816	1.414	1.251	
	104-109	106.66±1.453	2.517	2.359	P<0.025
	94-99	96.33±1.453	2.517	2.612	P<0.001

- Each mean result corresponds to a mean value of the three experiments.
- pHs are arranged in the table in a decreasing order (7.1, 5.4, 4.7) within each week.

S.E. = Standard error.

S.D. = Standard deviation.

Table (3): Changes in serum sodium potassium concentration (expressed as mmol/l) of juvenile Clarias lazera exposed to different treatment with H_2SO_4, pH_s 7.1 (control), 5.4 and 4.7 over a 12-week period.

Exposure period (weeks)	Range	Mean±S.E.	S.D.	% Coefficient of variation	Level of Significance
First week	1.90-2.10 4.30-4.40 4.70-4.85	2.000±0.081 4.366±0.033 4.783±0.044	0.141 0.058 0.076	7.071 1.322 1.597	 P<0.001 P<0.001
Second week	1.85-2.05 4.50-4.55 4.80-4.95	1.950±0.081 4.516±0.017 4.883±0.044	0.141 0.029 0.076	7.252 0.639 1.564	 P<0.001 P<0.001
Third week	2.00-2.10 4.65-4.75 4.95-5.05	2.050±0.041 4.700±0.029 5.000±0.029	0.071 0.050 0.050	3.449 1.064 1.000	 P<0.001 P<0.001
Fourth week	2.00-2.10 3.65-3.85 4.35-4.65	2.050±0.041 3.783±0.067 4.466±0.093	0.071 0.115 0.161	3.449 3.052 3.598	 P<0.001 P<0.001
Fifth week	2.30-2.35 3.70-3.75 4.05-4.20	2.325±0.020 3.733±0.017 4.117±0.044	0.035 0.029 0.076	1.521 0.773 1.855	 P<0.001 P<0.001
Sixth week	2.00-2.20 3.95-4.05 4.40-4.65	2.100±0.081 4.000±0.029 4.550±0.076	0.141 0.050 0.132	6.734 1.250 2.907	 P<0.001 P<0.001
Seventh week	2.55-2.80 3.60-3.70 4.00-4.25	2.675±0.102 3.717±0.073 4.100±0.076	0.177 0.126 0.132	6.608 3.385 3.226	 P<0.005 P<0.001
Eighth week	2.70-2.80 3.65-3.80 4.10-4.35	2.750±0.041 3.733±0.044 4.200±0.076	0.071 0.076 0.132	2.571 2.046 3.149	 P<0.001 P<0.001
Ninth week	2.40-2.65 3.50-3.60 3.80-3.90	2.525±0.102 3.550±0.029 3.850±0.029	0.177 0.050 0.050	7.001 1.408 1.299	 P<0.001 P<0.001
Tenth week	2.40-2.50 3.05-3.25 3.60-3.70	2.450±0.041 3.133±0.061 3.650±0.029	0.071 0.104 0.050	2.886 3.322 1.369	 P<0.001 P<0.001
Eleventh week	1.95-2.45 2.70-2.80 3.25-3.35	2.200±0.204 2.750±0.029 3.300±0.029	0.353 0.050 0.050	16.070 1.818 1.515	 N.S. P<0.01
Twelfth week	2.25-2.55 2.40-2.55 2.60-2.90	2.400±0.122 2.483±0.044 2.733±0.088	0.212 0.076 0.153	8.839 3.075 5.588	 N.S. N.S.

- Each mean result corresponds to a mean value of the three experiments.
- pH_s are arranged in the table in a decreasing order (7.1, 5.4, 4.7) within each week.

S.E. = Standard error.

S.D. = Standard deviation.

Table (4): Changes in serum calcium and chloride concentrations of juvenile *Clarias lazera* during exposure to acidified water (pH 3.1) over a 96-h period.

	Exposure period (hours)	Range	Mean $\pm$ S.E.	S.D.	% Coefficient of variation	Level of significance
Calcium (Ca^{2+})	Control	11.2-11.7	11.400$\pm$0.153	0.264	2.321	
(mg/dl)	24 h	10.0-10.4	10.167$\pm$0.120	0.208	2.047	$P < 0.005$
	48 h	12.4-12.6	12.500$\pm$0.058	0.100	0.800	$P < 0.005$
	72 h	10.9-11.2	11.067$\pm$0.088	0.153	1.380	N.S
	96 h	11.8-12.0	11.900$\pm$0.058	0.100	0.840	$P < 0.05$
Chloride (Cl^-)	Control	134-137	135.333$\pm$0.882	1.527	1.129	
(mmol/l)	24 h	117-120	118.466$\pm$0.867	1.501	1.267	$P < 0.001$
	48 h	104-106	105.166$\pm$0.601	1.041	0.989	$P < 0.001$
	72 h	80-83.7	82.066$\pm$1.089	1.888	2.300	$P < 0.001$
	96 h	56-58.1	57.080$\pm$0.615	1.065	1.866	$P < 0.001$

- Each mean result corresponds to a mean value of the three experiments.

S.E. = Standard error.

S.D. = Standard deviation.

When compared to control values 11.56 ± 0.032, changes induced in Ca^{+} ion concentration, due to chronic exposure of fish to acid, were significantly elevated throughout the duration of experiment (12 weeks) for both pH 5.4 (11.887 ± 0.009, $p < 0.001$) and pH 4.7 (12.060 ± 0.046, $p < 0.001$). Table (5).

With respect to chloride ion levels, significant decreasing values were observed throughout the chronic pH 5.4 exposure period.

12 week after exposure the Cl ion concentration decreased to 77.706 ± 0.633 with compared to control value 102.055 ± 0.066 At 4.7, the loss of Cl ions was somewhat more pronocenced which start and remained highly significant ($p < 0.001$) from the corresponding control values till the end of the experiment.Table (6). A dramatic decrease amounting to about 50 % loss was recorded during the last week of exposure giving a value of 54.46 ± 0.45 mmol/L.

II- In water samples:

Determination of whole body sodium, potassium and calcium ion efflux.

Flame photometric analysis of water electrolyte were performed on acid exposed (pH 3.1) and control (pH 7.1) fish to a certain that an electrolyte in balance is associated with acid exposure. were illustrated in Table 7 Sodium ion efflux was observed within 60 minutes showing a significant level of $p < 0.05$. A gradual continual efflux, ending with a highly significant maximum value of 65.04 ± 0.18 ppm ($p < 0.001$) when compared to control value (59.43 ± 0.08 ppm) was observed.

Potassium and calcium ions efflux showed a time dependent increase following the introduction of fish to pH 3.1 being significant only after 24 hours for potassium reaching a value of 10.86 ± 0.80 ppm ($p < 0.05$), comparable to the control value (8.18 ± 0.44 ppm), and after 45 minutes for calcium reaching a level of 64.00 ± 0.16 ppm ($p < 0.025$) from the untreated control level (61.66 ± 0.53 ppm).

Determination of the osmotic activity (osmolality)

Major changes in the osmolality levels of fish at both pH levels (5.4 and 4.7) occurred after chronic acid exposure as shown in table (Table 8). A substantial and rapid decrease was observed starting from the first week after treatment, recording values of 236.78 ± 0.949 and 22.05 ± 1.114 milliosmoles / kg for pH, 5.4 and 4.7 at the end of the experimental period (12 weeks), compared to their corresponding control value (272.77 ± 3.45 milliosmoles / kg.

Table (5) Changes in serum calcium concentration (expressed as mg/dl) of juvenile *Clarias lazera* exposed to different treatment with H_2SO_4, pH_s 7.1 (control), 5.4 and 4.7 over a 12-week period.

Exposure period (weeks)	Range	Mean±S.E.	S.D.	%Coefficient of variation	Level of Significance
First week	11.40-11.60	11.500±0.081	0.141	1.229	
	11.80-11.89	11.843±0.026	0.045	0.381	P<0.025
	12.48-12.60	12.530±0.036	0.062	0.498	P<0.001
Second week	11.40-11.50	11.450±0.041	0.071	0.617	
	12.04-12.12	12.086±0.024	0.042	0.344	P<0.001
	13.69-13.72	13.703±0.009	0.015	0.115	P<0.001
Third week	11.10-11.30	11.200±0.081	0.141	1.263	
	12.81-12.89	12.853±0.023	0.040	0.314	P<0.001
	14.02-14.13	14.083±0.033	0.057	0.404	P<0.001
Fourth week	10.90-11.00	10.950±0.041	0.071	0.646	
	12.89-12.96	12.917±0.022	0.038	0.293	P<0.001
	13.84-13.91	13.873±0.020	0.035	0.253	P<0.001
Fifth week	10.80-11.30	11.050±0.204	0.353	3.199	
	11.89-11.98	11.936±0.026	0.045	0.038	P<0.025
	13.28-13.32	13.300±0.011	0.020	0.150	P<0.001
Sixth week	11.54-11.60	11.570±0.024	0.042	0.367	
	11.47-11.57	11.520±0.029	0.050	0434	N.S.
	12.01-12.09	12.043±0.024	0.042	0346	P<0.001
Seventh week	11.41-11.56	11.485±0.061	0.106	0.923	
	11.54-11.60	11.577±0.018	0.032	0.278	N.S.
	12.30-12.44	12.373±0.040	0.070	0.566	P<0.001
Eighth week	11.60-11.70	11.650±0.040	0.070	0.607	
	11.87-11.96	11.913±0.026	0.045	0.378	P<0.01
	12.86-12.94	12.890±0.023	0.040	0.313	P<0.001
Ninth week	11.85-11.90	11.875±0.020	0.035	0.298	
	12.10-12.17	12.137±0.020	0.035	0.289	P<0.001
	12.89-12.93	12.906±0.012	0.021	0.161	P<0.001
Tenth week	11.30-11.46	11.380±0.065	0.113	0.994	
	12.26-12.34	12.303±0.023	0.040	0.328	P<0.001
	12.87-12.98	12.930±0.032	0.056	0.431	P<0.001
Eleventh week	11.26-11.38	11.320±0.049	0.085	0.749	
	11.94-12.00	11.966±0.018	0.030	0.255	P<0.001
	12.59-12.69	12.640±0.050	0.398	0.029	P<0.001
Twelfth week	11.52-11.60	11.560±0.032	0.056	0.489	
	11.87-11.90	11.887±0.009	0.015	0.128	P<0.001
	12.00-12.15	12.060±0.046	0.079	0.658	P<0.001

- Each mean result corresponds to a mean value of the three experiments.
- pH_s are arranged in the table in a decreasing order (7.1, 5.4, 4.7) within each week.

S.E. = Standard error.

Table (6): Changes in serum chloride concentration (expressed as mmol/l) of juvenile <u>Clarias</u> <u>lazera</u> exposed to different treatment with H_2SO_4, pH_s 7.1 (control), 5.4 and 4.7 over a 12-week period.

Exposure period (weeks)	Range	Mean±S.E.	S.D.	%Coefficient of variation	Level of Significance
First week	100-104 88-90 75-79	102.000±1.633 89.203±0.612 77.000±1.154	2.828 1.602 2.000	2.773 1.188 2.597	 P<0.005 P<0.001
Second week	100-103 87-89.1 74-76	101.500±1.224 87.780±0.664 75.320±0.663	2.121 1.149 1.149	2.089 1.309 1.525	 P<0.001 P<0.001
Third week	99.60-100.13 89.90-91.40 73.00-73.90	99.865±0.216 90.520±0.452 73.510±0.236	0.375 0.783 0.462	0.375 0.865 0.628	 P<0.001 P<0.001
Fourth week	94.90-97.10 93.81-96.03 70.84-73.46	96.000±0.898 95.160±0.684 72.013±0.768	1.556 1.185 1.331	1.620 1.245 1.848	 N.S. P<0.001
Fifth week	95.00-97.00 93.22-99.32 78.89-81.04	96.000±0.816 96.410±1.766 80.040±0.625	1.414 3.059 1.083	1.473 3.173 1.354	 N.S. P<0.001
Sixth week	99.85-100.00 99.75-100.10 87.54-88.22	99.925±0.061 99.970±0.111 87.960±0.213	0.106 0.197 0.369	0.106 0.192 0.419	 N.S. P<0.001
Seventh week	101.00-101.19 85.67-88.50 74.13-75.40	101.095±0.077 86.780±0.871 74.870±0.383	0.134 1.508 0.664	0.133 1.738 0.886	 P<0.001 P<0.001
Eighth week	102.11-102.71 80.70-84.74 61.61-64.89	102.410±0.245 82.610±1.171 62.980±0.984	0.424 2.028 1.705	0.414 2.455 2.707	 P<0.001 P<0.001
Ninth week	102.76-103.35 81.13-85.20 60.64-60.91	103.055±0.241 83.640±1.268 60.800±0.083	0.417 2.197 0.144	0.405 2.626 0.236	 P<0.001 P<0.001
Tenth week	100.59-101.69 82.00-83.03 58.65-59.74	101.140±0.202 82.490±0.298 59.136±0.320	0.778 0.516 0.554	0.769 0.625 0.937	 P<0.001 P<0.001
Eleventh week	100.18-100.59 79.91-81.62 54.20-55.11	100.385±0.028 80.510±0.554 54.540±0.287	0.289 0.959 0.497	0.289 1.192 0.911	 P<0.001 P<0.001
Twelfth week	101.74-102.37 76.99-78.97 53.55-54.96	102.055±0.066 77.706±0.633 54.460±0.459	0.445 1.097 0.795	0.436 1.412 1.458	 P<0.001 P<0.001

- Each mean result corresponds to a mean value of the three experiments.
- pH_s are arranged in the table in a decreasing order (7.1, 5.4, 4.7) within each week.
S.E. = Standard error.
S.D. = Standard deviation.

Table (7): Changes in whole body sodium, potassium and calcium ions efflux (expressed as ppm) of juvenile *Clarias lazera* exposed to acidified water(pH 3.1, TLm value) over a 24-h period.

Exposure Time	Ion efflux values					
	Sodium (Na^+)		Potassium (K^+)		Calcium (Ca^{2+})	
	Mean control values ±S.E	Mean treated values ± S.E	Mean control values ±S.E.	.Mean treated values ± S.E.	Mean control values ±S.E.	Mean treated values ± S.E.
0 min	56.016±0.140	56.178±0.142	6.535±0.112	6.535±0.794	60.833±0.772	60.833±0.224
15 min	56.097±0.092	56.747±1.010	6.614±0.197	6850±0.894	60.833±0.736	62.005±0.335
30 min	56.910±0.211	57.723±0.968	6.850±0.221	7.165±0.883	61.666±0.711	62.055±0.087
45 min	57.154±0.134	59.105±0.911	7.086±0.168	7.244±0.646	61.666±0.534	64.000±0.169 [a]
60 min	57.723±0.088	59.593±0.584 [a]	7.322±0.089	7.480±0.872	66.666±0.421	66.666±0.156
2 h	58.048±0.110	61.544±0.666 [b]	7.559±0.342	7.559±0.967	66.666±0.411	66.666±0.311
3 h	58.861±0.243	61.788±0.789 [a]	7.559±0.021	8.031±0.873	67.005±0.672	68.333±0.071
4 h	59.186±0.097	62.195±0.594 [b]	7.874±0.167	8.031±0.734	68.333±0.495	70.833±0.376 [a]
24 h	59.430±0.081	65.040±0.183 [b]	8.188±0.442	10.866±0.801 [a]	70.833±0.663	74.166±0.258 [b]

- Each result corresponds to a mean value of the three experiments.
S.E. = Standard error.
- Control pH = 7.1 ± 0.1.

Table (8): Changes in serum osmoticactivity (osmolality) (expressed as milliosmoles/Kg) ofjuvenile Clarias lazera exposed to different treatment with H_2SO_4, pH. 7.1 (control), 5.4 and 4.7 over a 12-week period.

Exposure period (weeks)	Range	Mean±S.E.	S.D.	% Coefficient of variation	Level of Significance
First week	268.54-277.00	272.770±3.454	5.982	2.193	
	240.72-242.01	241.313±0.376	0.651	0.269	P<0.001
	232.32-237.34	235.050±1.466	2.539	1.080	P<0.001
Second week	267.02-268.60	267.810±0.645	1.117	0.417	
	237.00-239.04	237.727±0.658	1.139	0.479	P<0.001
	200.94-205.44	202.513±1.465	2.537	1.253	P<0.001
Third week	250.67-258.66	254.665±3.261	5.649	2.218	
	230.06-234.12	231.807±1.206	2.088	0.901	P<0.005
	168.31-169.58	168.867±0.375	0.649	0.384	P<0.001
Fourth week	267.86-273.38	270.620±2.253	3.903	1.442	
	219.86-222.14	221.243±0.702	1.215	0.549	P<0.001
	155.44-164.94	159.490±2.830	4.902	3.074	P<0.001
Fifth week	268.13-271.60	269.865±1.417	2.454	0.909	
	251.60-253.53	252.796±0.603	1.045	0.413	P<0.001
	221.18-231.10	226.506±2.887	5.005	2.208	P<0.001
Sixth week	264.27-268.54	266.405±1.743	3.019	1.133	
	257.94-259.18	258.693±0.382	0.662	0.256	P<0.025
	210.18-216.27	213.587±1.795	3.109	1.455	P<0.001
Seventh week	279.33-290.14	284.735±4.413	7.644	2.684	
	263.53-268.20	265.833±1.348	2.336	0.879	P<0.025
	230.96-235.02	233.093±1.176	2.038	0.874	P<0.001
Eighth week	250.67-254.04	252.350±1.376	2.383	0.944	
	258.20-262.82	260.233±1.362	2.359	0.906	P<0.025
	243.38-247.80	245.366±1.295	2.243	0.914	P<0.025
Ninth week	273.38-277.40	275.390±1.641	2.842	1.032	
	248.71-251.18	249.660±0.765	1.326	0.531	P<0.001
	237.08-225.98	238.783±0.891	1.544	0.646	P<0.001
Tenth week	262.35-265.35	263.850±1.224	2.121	0.804	
	240.00-243.18	241.966±0.992	1.719	0.710	P<0.001
	223.67-225.98	224.606±0.702	1.215	0.541	P<0.001
Eleventh week	261.77-262.82	262.295±0.428	0.742	0.283	
	239.18-243.81	241.473±1.337	2.315	0.959	P<0.001
	220.70-224.68	222.440±1.176	2.036	0.905	P<0.001
Twelfth week	252.17-255.22	253.695±1.245	2.157	0.850	
	234.94-238.17	236.786±0.949	1.643	0.694	P<0.001
	220.60-224.24	222.050±1.114	1.929	0.869	P<0.001

- Each mean result corresponds to a mean value of the three experiments.
- pH. are arranged in the table in a decreasing order (7.1, 5.4, 4.7) within each week.

S.E. = Standard error.

S.D. = Standard deviation.

DISCUSSION

Exposure of Clarias lazera in the present study to acute acid stress, led to a pronounced drop in serum Na^+ and Cl- levels. Serum Nå and Cl- depletion as a result of acute exposure has been domesterated for a variety of species such as yellow Perch flavescens (Lyons, 1982), large scale sucker Catostomus macrocheilus (McKeown et al, 1985), brook trout Salvelinus fontinalis (Packer and Dunson, 1970), rainbow trout salmon Salmo salar (McWilliams, 1980 b, and Booth et. al., 1982), juvenile rainbow trout (Barton et. al., 1985) and juvenile salmonids (Weiner, 1984 and Wood et al, 1988 a&b).

The decline in serum Na^+ and Cl- concentrations were detected after exposure of Clarias lazera to chronic acid stress (pH_5 5.4 and 4.7). This is in agreement with a previous studies by Leivestad, et al. (1976) and Mc Williams (1980 b).

The following study also indicates that the lower pH 4.7 appears to have a greater significant effect on both electrolytes than the much higher pH 5.4 level (Mc Donald et al., 1983 a, and Packer and Dunson, 1972).

There have been a number of pervious studies on the chronic ion regulatory effects (2 weeks to 3 months) of low pH exposure (Mc Williams, 1980 a ; Lee et. al., 1983; Brown et. al., 1986 b; Weiner et. al., 1986; Jones et. al., 1987 and Audet et. al., 1988).

The reduced uptake of Na^+ in acid waters was explained to be due to an increased load on the Na+ pump mechanism as a consequence serum ionic levels were decreased. Cl- loss during acute and chronic acid exposure may be a result of passive Cl- efflux that exceeded active uptake.

Increased serum K^+ resulting from exposure to environmental acidification has been observed for rainbow trout at pH_4 (Mc Donald et. al., 1980 α Booth et. al. 1982, and for carp Cyprinus carpio at pH_4, but not at pH 5.1 (Ultsch et. al., 1981) and Wood et. al., 1988 b).

Clarias lazera exposed to low pH of 5.4 and 4.7 during chronic experiments showed a significant increase in serum K+ level, followed by a gradual recovery to the control at the end of the experimental period. Rainbow trout Salmo gairdneri exposed to low pH 4.9 and 5.6 (McKeown et. al., 1985) behaved some what similar to Clarias lazera. Experiments of Leivestad et. al., 1976 and Mc Donald andWood, 1981) reported an increased loss of K^+ from muscle tissue at low pH, which might account for serum increases in K^+. A recovery showed during the chronic experiment may be due to that fish were partially restoring the ion level by time either by partial reduction of extracellular space, breakdown of erthrocytes thus releasing K^+ or releasing K^+ from cells buffering H^+ to maintain normal cell's electrical potential.

The present data indicate a slight but significant increase in serum calcium levels during acute exposure. This was in accordance with data obtained using Oreochromis mossambicus exposed to pH 4, Wendelaar Bonga et. al., (1984) related this elevation to the indirect effect of the enhanced prolactin secretion resulting in a significant hypercalcemia that was also observed after 2 weeks of acid exposure. In Tilipia, prolactin stimulates the active uptake of Calcium through the gills and has a hypercalcemic action (Wendelaar Bonga and Flik, 1982 and Wendelaar Bonga et. al., 1984).

The increase of calcium level caused by chronic exposure to low pH_s (5.4 and 4.7) in the present study, has been previously observed in other fish species (Holmes and Donaldson, 1969; Whitehead et. al., 1978). Saunders et. al., (1983) who observed high calcium level in Salmon Salmo salar reared at low pH during much of the experiment (5 months), and suggested that this elevation may be an indication of calcium mobilization from skeletal compartments, impaired renal excretion of calcium, elevated calcium uptake from brachial or gut regions, or some combination of these mechanisms. However, it was not clear from the study which, if any, of these mechanisms was responsible for the elevated calcium level.

In the present investigation, acute acid exposure (pH 3.1) for 24 hour was observed to provoke major alterations in ionic balance in Clarias lazera.

Along list of studies (Packer and Dunson, 1970 and 1972; Maetz, 1973; Ashcom, 1979; McWilliams, 1982b; McDonald, 1983 b; Hobe et al; 1984a, Audet and Wood, 1988; Wood 1988 and Wood et al., 1990 showed that exposure of fish to external acidic pH results in an inhibition of active sodium influx and a stimulation of its diffusional efflux, leading to net sodium loss and reduction of body Na levels.

With regards to potassium ion concentration in acutely exposed Clarias lazera to acid 3.1, although serum K concentration was significantly increased after 24 hours, potassium efflux was also found to increase during the efflux experiments, but values were significantly higher only at the final sample (24 hours). These findings suggest that Clarias lazera was capable of either maintaing or restoring serum potassium concentration if only on this short term faces, through some adaptive mechanism. Similar results were demonstrated previously by Booth et. al., 1982.

With respect to the calcium ion reported in the present study on Clarias lazera exposed to pH 3.1, initially calcium efflux was nonsignificant compared to their corresponding controls, until 45 minutes of exposure where Ca efflux became significantly different from controls.

Low pH (4.8) treatment for 24 hours also stimulated Ca efflux in rainbow trout Salmo gairdneri (Reid and McDonald, 1988). Such changes in Ca permeability may also account for most of the drop in plasma Ca following acute exposure.

Several mechanisms have been proposed to explain the effect of H^+ ion on ionic efflux, but most are encompassed by increased brachial permeability to water and ions (McDonaled, 1983 -b, Lee et. al., 1983; and Fryer et. al., 1988).

At both pH_5 5.4 and 4.7 in the present chronic exposure systems, serumosmolality dropped abruptly during the first four weeks. The reduction was more severe at pH 4.7. After 4 weeks a gradual increase followed in both pH groups which again showed progressive decreases after 7 weeks at pH 5.4 and 9 weeks at pH 4.7 until termination of the experiment. The initial drop and successive return of osmolality level has been repeatedly reported in different fish species, for different durations (14 - 52 days) and at different pH level (4.5 - 5.5)(Giles et. al., 1984; McKeown et. al., 1985; Brown et. al., 1986 b; Jones et. al., 1987; Audet et. al., 1988 and Fryer et. al., 1988).

The present data obtained on serum osmolality level in Clarias lazera chronically exposed to low pH reinforce the view that reduced pH enhances that ion loss that fish in freshwater typically experience, and that osmolality as a related parameter provide some additional insight into the significance of the increased efflux of ions due to gill permeability changes.

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