The Pharma Innovation Journal 2020; 9(6): 306-310

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(6): 306-310

www.thepharmajournal.com
Received: 06-04-2020
Accepted: 07-05-2020

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Serum biochemistry in experimentally induced sudden death syndrome in broiler chickens

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Abstract
In the present study, a total of eighteen different farms with the history of sudden death syndrome (SDS) were investigated. Analysis of data revealed that the susceptibility age of sudden death syndrome was found to be as early as first week and age between 4 to 5weeks highly susceptible. The average SDS mortality percentage in 18 broiler farms ranged from 0.61 to 2.49 per cent. Clinical signs, gross and histopathological changes have been recorded from field cases. SDS was induced by administration of lactic acid through crop intubation. Clinical signs observed included loss of balance, abruptly falling on to the ground, violent wing-flapping, strong muscular contractions and extended neck and legs. Majority of affected broilers were found dead lying on their backs. Serum sodium levels in the SDS birds were significantly decreased in treatment group birds. Potassium, Calcium, Magnesium, Phosphorus, LDH and CPK levels were significantly increased in treatment group when compared to control.

Keywords: SDS, broiler, serum biochemistry, age

Introduction
Sudden death syndrome (Acute Death Syndrome, heart attack, flip-over disease or died in good condition) is a condition in which healthy broiler chickens die suddenly for no discernible cause. It was first described as "Edema of lungs" in England. Later was reported in Eastern Europe, Canada, Australia and United States.
Broilers that die of SDS show no specific abnormalities. They are usually male, appear healthy and often above average flock body weight. Prior to death they appear normal and are often seen feeding, drinking or walking. Birds exhibit neck and leg extension, squawk, wing beating and convulsions prior to death, most of the times affected broilers are found dead lying on their backs. As a result, the condition often been referred to as "Flip-Over Disease" and most poultry farmers describe the condition as "heart attack". Death occurs within minutes however, birds can be revived if they are vigorously massaged during the early stages of the attack. Sudden death syndrome has developed into a major problem to the broiler industry in many parts of the world. Broilers of all ages are affected starting as early as 2 days of age and through to market age. But peak mortality usually occurs between 3 and 4 weeks of age.

Materials and Methods
Two week old broiler chicks of Vencobb strain (80 numbers) were used for the experimental study. The birds were obtained from a commercial hatchery unit, Bangalore. The study was approved by Institutional Animal Ethics committee (IAEC).
Commercially available lactic acid solution 85% (pure form) obtained from SISCO research Lab. Pvt. Ltd. Mumbai, was used in the present study. Sodium, potassium and calcium kits from Lab-Care Diagnostics Pvt. Ltd. Sarigam, India. Magnesium and phosphorus kits from Crest Bio-systems, Goa, India. LDH and CPK kits were purchased from Transasia Bio-Medicals Ltd. Were used for the study.
Two week old broiler chicks (80 numbers) procured from a reputed breeding farm were reared under optimum managemental condition during entire period of study. All birds were provided with broiler starter feed and water *ad libitum*. The birds were kept for acclimatization in experimental house, at Department of Pathology for 15 days.
A pilot study was conducted on four week old broiler birds (42 numbers) to fix the dose of calcium lactate and lactic acid that cause death.

a) During the study, calcium lactate solution was administered at different concentration viz., 5, 10, 15, 20 and 30 per cent through crop intubation and the results were systematically recoded.
b) Lactic acid (85%) solution was administered at different dilutions viz., 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 85 per cent through crop intubation at different time intervals. Time taken for mortality pattern was systematically recorded.

Based on the occurrence of mortality in broiler birds with clinical manifestation of sudden death syndrome upon administration of lactic acid solution (85%), the dosage of lactic acid solution was fixed to induce sudden death syndrome in broilers

Experimental Protocol

The birds were divided in to treatment (Group 1) and control group (Group 2) as shown in the Table 1. Treatment group 1 was further replicated in to 1A, 1B and 1C consisting of six birds in each group. Group 1A, 1B and 1C were given lactic acid solution (85%) at the dose rate of 10ml per bird through crop intubation at the time interval of one hour each. The group 2 birds were administered 10 ml distilled water for each bird at the time interval of one hour each. During the period of experiment, the birds belonging to treatment and control groups were observed for development of clinical signs, feed and water consumption.

Table 1: classification of the treatment (Group 1) and control group (Group 2)

Group 1	Treatment	Dose	No. of birds
1A	Lactic acid (extra pure) 85%	10 ml	6
1B	Lactic acid (extra pure) 85%	10 ml	6
1C	Lactic acid (extra pure) 85%	10 ml	6
Group 2	**Control group**		
2A	Distilled water	10 ml	6
2B	Distilled water	10 ml	6
2C	Distilled water	10 ml	6

The blood samples were collected directly from the heart of dead birds (within a minute after the death). The blood samples obtained were allowed to clot and centrifuged at 1500 rpm for 5 min to separate the serum. The sera samples were stored at -200 C for biochemical estimation.

The lactate dehydrogenase (LDH-P) and Creatinine Phosphokinase (CPK) activities in the serum were measured as per the method of DGKC (Deutsche Gesellschaft für klinische Chemie) and UV-Kinetic method respectively using semi-automatic analyser.

The sodium and potassium were measured by using colorimetric method and calcium was measured by method of Metallochromogen Arsenazo III. The magnesium and phosphorus were measured by Calmagite method and Molybdate U.V. method respectively. The data generated from different parameters of the experimental study were subjected to statistical analysis. The results were subjected to one way analysis of variance (ANOVA) test using Graph pad Prism Version 5 for Windows.

Results

SDS was experimentally reproduced in 4 week old broilers by administration of lactic acid solution (85%) at the dose rate of 10ml per bird through crop intubution at the time interval of one hour each. The details pertaining to mortality pattern, clinical signs, gross and histological lesions observed in treatment and control group has been presented as here under.

Table 2: Administration of Lactic acid (85%) solution to 4 week old broilers at different concentrations through crop intubation route

Percentage of lactic acid	Dose of lactic acid	Time of inoculation	Time of Mortality
5%	5 ml	2 pm	No mortality
10%	5 ml	2.05 pm	No mortality
15%	5 ml	2.10 pm	No mortality
20%	5 ml	2.25 pm	No mortality
30%	5 ml	2.30 pm	No mortality
40%	5 ml	2.35 pm	No mortality
50%	5 ml	2.40 pm	No mortality
60%	10 ml	2.45 pm	No mortality
70%	10 ml	2.50 pm	No mortality
80%	10 ml	3 pm	No mortality
85% (pure form)	10 ml	3.05 pm	Mortality at 4pm

A total of 18 birds comprising of six birds in each group were administered 85 per cent lactic acid solution at the dose rate of 10 ml/ bird through crop intubution. All six birds in each group died showing clinical signs of sudden death syndrome at the end of 55 minutes. Whereas the control group birds administered with distilled water did not show any mortality during this period. The details of this study have been presented in the table 3.

Table 3: Mortality pattern of experimentally induced sudden death syndrome in 4 week old broiler chicken

Treatment group 1	Treatment	No. of birds treated	Mortality pattern	
			No. of birds died	Duration of time
1A	Lactic acid (85%)	6	6	55 minutes
1B	Lactic acid (85%)	6	6	55 minutes
1C	Lactic acid (85%)	6	6	55 minutes
Control Group 2				
2A	Distilled water	6	6	No mortality
2B	Distilled water	6	6	No mortality
2C	Distilled water	6	6	No mortality

Serum Biochemistry

The mean ±SE values of serum sodium, potassium, calcium, magnesium and phosphorus observed in control and treatment groups are presented in Table 4.

Table 4: Mean (± SE) serum biochemical values of broiler chickens affected with Sudden Death Syndrome (n=36)

Parameters	Control group 2A	Treatment group 1A	Control group 2B	Treatment group 1B	Control group 2C	Treatment group 1C
Sodium (mmol/L)	143.7±0.32^{a}	135.3±0.65^{b}	143±0.43^{a}	134.4±0.44^{b}	143±0.40	134.5±0.33^{b}
Potassium (mmol/L)	3.37±0.08^{a}	7.57±0.15^{b}	3.58±0.13^{a}	7.61±0.17^{b}	3.69±0.07^{a}	7.42±0.12^{b}
Calcium (mg/dl)	10.74±0.15^{a}	14.62±0.21^{b}	10.70±0.09^{a}	14.56±0.13^{b}	10.74±0.69^{a}	14.47±0.14^{b}
Magnesium (meq/L)	4.18±0.07^{a}	6.18±0.04^{b}	4.30±0.06^{a}	6.37±0.09^{b}	4.27±0.05^{a}	6.45±0.10^{b}
Phosphorus (mg/dl)	5.22±0.03^{a}	11.02±0.11^{b}	5.39±0.09^{a}	11.34±0.11^{b}	5.43±0.08^{a}	11.34±0.10^{b}
LDH(IU/L)	1034±22.99^{a}	2274±62.42^{b}	1084±24.17^{a}	2342±19.10^{b}	1064±16.42^{a}	2354±18.14^{b}
CPK(IU/L)	627.3±8.01^{a}	1138±13.45^{b}	638.0±7.15^{a}	1121±28.48^{b}	641.8±4.40^{a}	1156±14.05^{b}

Mean value bearing different superscripts differ significantly ($P< 0.05$)

The mean ± SE concentration of sodium was 135.3 ± 0.65, 134.4 ± 0.44, 134.5 ± 0.33 and 143.7 ± 0.32, 143 ± 0.43, 143 ± 0.40 mmol/lt respectively in treatment group 1A, 1B and 1C and control groups 2A, 2B and 2C. The sodium concentration showed no significant difference among the treatment groups. The sodium concentration was significantly low (P<0.05) in treatment groups when compared to control groups (Table 4, Fig 1).

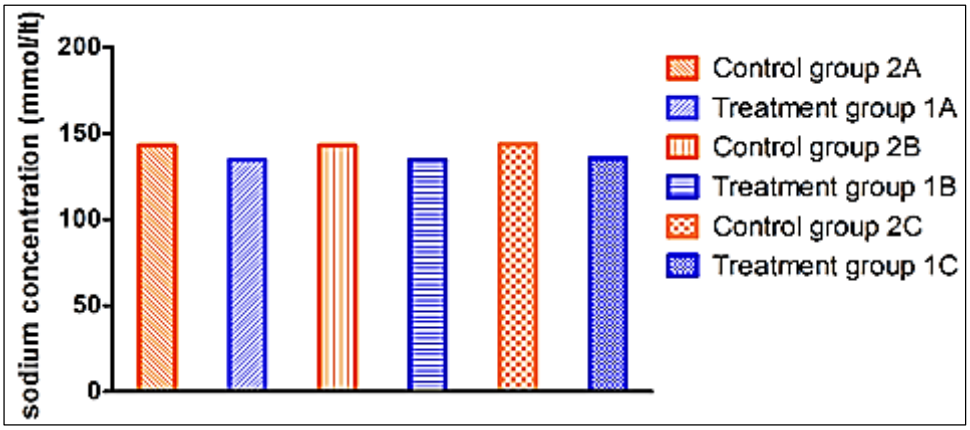

Fig 1: The mean serum sodium (mm l/lt) values of control and treatment group birds

The mean± SE concentration of potassium was 7.57±0.15, 7.61±0.17, 7.42±0.12 and 3.37±0.08, 3.58±0.13, 3.69±0.07 mmol/lt respectively in treatment group 1A, 1B and 1C and control group 2A, 2B and 2C. The potassium concentration was similar and showed no significant difference among the treatment groups. The potassium concentration was significantly high (P<0.05) in treatment groups when compared to control groups (Table 4, Fig. 2).

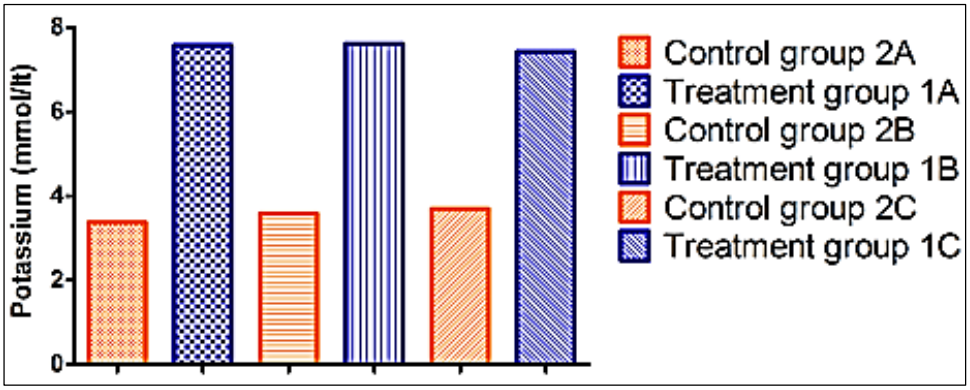

Fig 2: The mean potassium (mmol/lt) values of control and treatment group birds

The mean± SE concentration of calcium was 14.62±0.21, 14.56±0.13, 14.47±0.14 and 10.74±0.15, 10.70±0.09, 10.74±0.69 mg/dl respectively in treatment group 1A, 1B and 1C and control group 2A, 2B and 2C. The calcium level was similar and showed no significant difference among the treatment groups. The sodium concentration was significantly high (P<0.05) in treatment groups when compared to control groups (Table 4, Fig. 3).

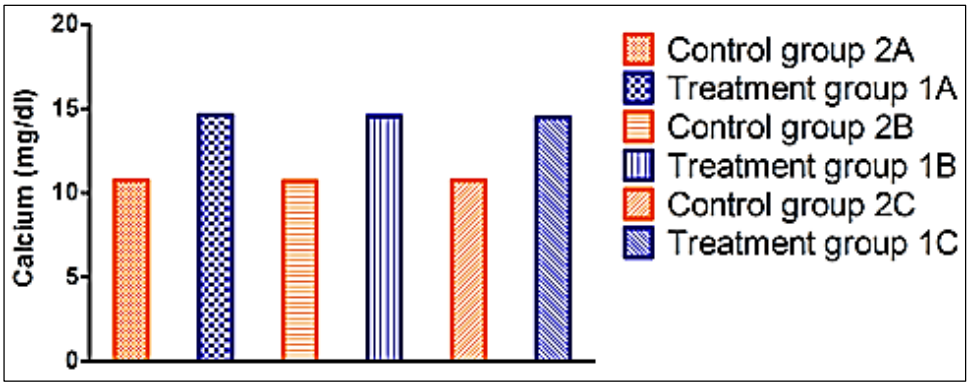

Fig 3: The mean calcium (mg/lt) values of control and treatment group birds

The mean± SE concentration of magnesium was 6.18±0.04, 6.37±0.09, 6.45±0.10 and 4.18±0.07, 4.30±0.06, 4.27±0.05 meq/lt respectively in treatment group 1A, 1B and 1C and control group 2A, 2B and 2C. The level of magnesium showed no significant difference among the treatment groups. The magnesium concentration was significantly high (P<0.05) in treatment groups when compared to control groups (Table 4, Fig.4).

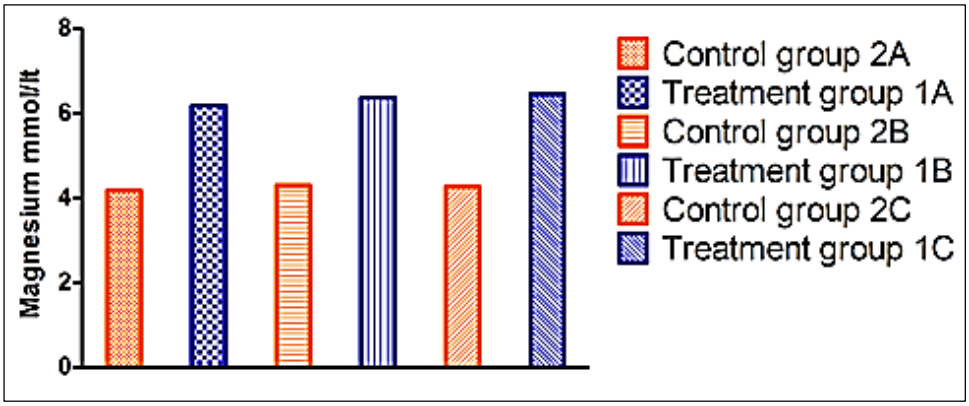

Fig 4: The mean magnesium (meq/lt) values of control and treatment group birds

The mean ± SE concentration of phosphorus was 11.02±0.11, 11.34±0.11, 11.34±0.10 and 5.22±0.03, 5.39±0.09, 5.43±0.08 mg/dl respectively in treatment group 1A, 1B and 1C and control group 2A, 2B and 2C. The level of phosphorus showed no significant difference among the treatment groups. The phosphorus concentration was significantly high (P<0.05) in treatment groups when compared to control groups (Table 4, Fig 5).

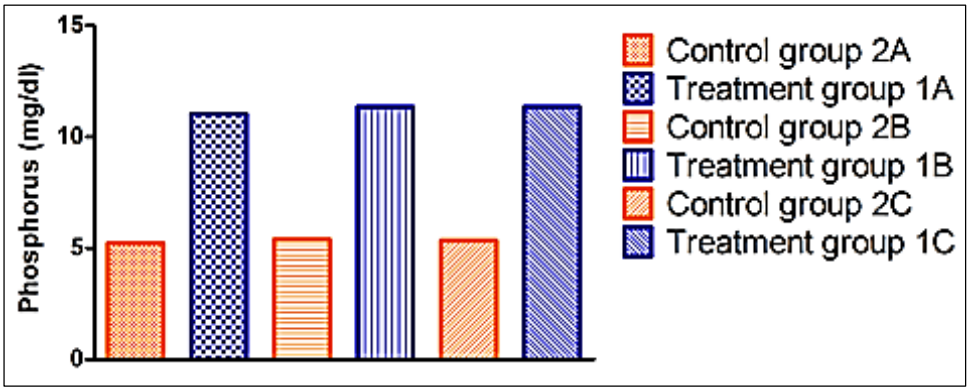

Fig 5: The mean phosphorus (mg/dl) values control and treatment group birds

The mean ±SE concentration of LDH was 2274 ±62.42, 2342 ±19.10, 2354 ±18.14 and 1034 ±22.99, 1084 ±24.17, 1064 ±16.42 IU/lt respectively in treatment group 1A, 1B and 1C and control group 2A, 2B and 2C. The level of LDH showed no significant difference among the treatment groups. The LDH concentration was significantly high (*P*<0.05) in treatment groups when compared to control groups (Table 4, Fig. 6)

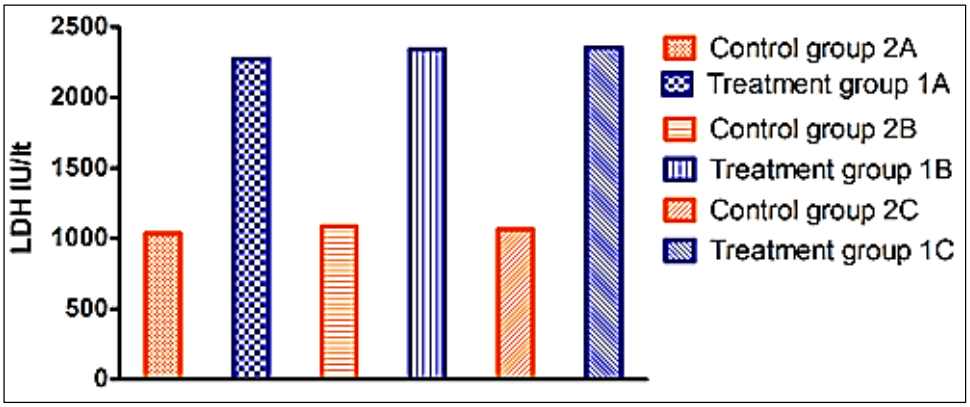

Fig 6: The mean LDH (IU/lt) values of control and treatment group birds

The mean ± SE concentration of CPK was 1138±13.45, 1121±28.48, 1156±14.05 and 627.3±8.01, 638.0±7.15, 641.8±4.40 IU/lt respectively in treatment group 1A, 1B and 1C and control group 2A, 2B and 2C. The level of CPK showed no significant difference among the treatment groups. The CPK concentration was significantly high (P<0.05) in treatment groups when compared to control groups (Table 4 Fig. 7).

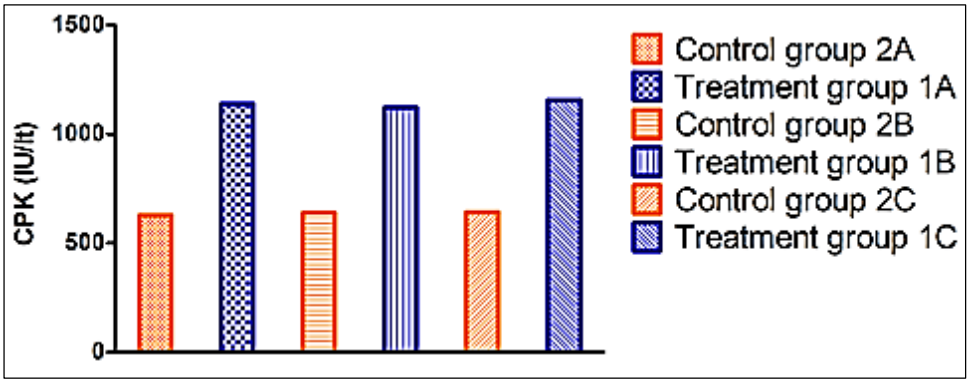

Fig 7: The mean CPK (IU/lt) values of control and treatment group birds

Discussion

Biochemical parameters like serum sodium, potassium, calcium, magnesium, phosphorus, LDH and CPK in experimentally induced sudden death syndrome have been discussed as here under.

In the present study, serum sodium level in the SDS affected birds was significantly decreased when compared to control birds. This observation agrees with the findings of Riddel and Orr (1980) [4]; Rotter *et al*. (1985) [5] and Noriaki Imaeda, 1999. The increase in the mean serum sodium level may be due to the movement of ions between the erythrocytes and sera after death as rightly indicated by Riddel and Orr (1980) [4]; Rotter *et al*. (1985) [5] and Noriaki Imaeda (1999) [2].

The level of potassium, calcium, magnesium, phosphorus, LDH and CPK were significantly increased in treatment group when compared to control group birds. Similar change was observed by Riddel and Orr (1980) [4] and Noriaki (1999) [2] and concluded that changes in level of potassium after death was attributed to movement of ions between the erythrocytes and sera (Riddel and Orr, 1980) [4]. Rotter *et al*. (1985) [5] reported that statistically significant decrease in K^+ content of heart tissue of SDS birds. SDS associated with myocardial abnormalities due to electrolyte imbalance produces ventricular myocardial degeneration, necrosis and fibrosis (Pass, 1983) [3]. In the present study, electrolyte imbalance between sodium and potassium was consistently observed in the treatment groups and this could leads to ventricular arrhythemia followed by myocardial abnormalities like degeneration and necrosis in sudden death syndrome cases.

Similar change was also observed by Riddel and Orr (1980) [4] who reported increased magnesium level in the birds affected with sudden death syndrome and attributed changes in level of magnesium to movement of ions between the erythrocytes and sera. Similar change was also observed by Riddel and Orr (1980) [4] and attributed changes in level of phosphorus after death to movement of ions between the erythrocytes and sera.

Masashi Kawada *et al*. (1994) reported that pathological changes relevant to SDS in the heart was due to myocardial necrosis and atherosclerotic lesions in the aorta. They also opined that LDH was derived from degenerated cardiac and arterial myocytes in the heart. Noriaki Imaeda (1999) [2] reported higher LDH activity in 4 to 9 week old SDS birds than in healthy birds. The increased levels of LDH and CPK could be due to pathological changes relevant to SDS in the heart- myocardial necrosis and atherosclerotic lesions. LDH and CPK were derived from degenerated cardiac and arterial myocytes in the heart. (Noriaki Imaeda, 1999) [2]. In the present study, the lesions observed in the heart could be responsible for increased LDH and CPK.

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