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*Full Length Article*

# Evaluation of Relative Resuscitative Effects of Hypertonic and Isotonic Saline Solutions as an Adjunct to Ceftiofur HCl in Bovine Neonatal Diarrhea Associated with *Escherichia coli*

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## Abstract

This study was conducted for evaluation of relative efficacy of hypertonic saline (HSS) and isotonic saline (ISS) solutions in neonatal diarrhea associated with *Escherichia coli* (*E. coli*) in buffalo calves. For this purpose, neonatal diarrhea was induced in 24 healthy male buffalo calves through oral administration of eneteropathogenic *E. coli* (K99). After 12 h of successful induction of diarrhea, the calves were randomly divided into two equal groups viz. A and B ($n$=12 each). Group A was treated with ISS @ 90 mL/kg body weight (BW), while calves of group B were administered HSS @ 4 mL/kg BW. Both groups were additionally treated with ceftiofur HCl and flunixin meglumine @ 6 and 2 mg/kg BW, IM, respectively. The relative efficacy of two treatments was evaluated on the basis of clinical parameters, hematological analyses, hemodynamic parameters, blood gas analyses and serum electrolytes. All parameters were recorded at baseline (before induction of diarrhea), during diarrhea, t=1, t=3, t=6, t=12, t=24 and t=36 h after treatment. The calves treated with HSS (group B) resuscitated from neonatal diarrhea more rapidly (within 24 h) and effectively than ISS (group A) and showed significant ($P < 0.05$) improvement. At t=24 h, values for mean arterial pressure, central venous pressure and arterial oxygen delivery were 108.6±9.8 and 116.3±14.2, 12±1.4 and 15.5±1.5 and 69.3±3.3 and 85.5±3.7 in groups A and B, respectively. Calves of group B also experienced decrease in hematocrit and hemoglobin concentration as well as increases in blood pH, bicarbonate concentration and serum electrolytes. On the basis of findings of this study, it was concluded that HSS offsets deleterious hemodynamic effects of hypovolemia, improves oxygenation, corrects metabolic acidosis and increases survival in *E. coli* associated neonatal diarrhea of buffalo calves which makes it more practical and economical alternative to the use of large volumes of isotonic saline solution for use in field settings. 

**Keywords:** Hypovolemia; Dehydration; Acidosis; Induction; Neonatal Diarrhea; Hypertonic saline

## Introduction

Neonatal diarrhea caused by *E. coli* and other gut associated microorganisms is a serious welfare problem and is considered one of the main causes of calf death worldwide and also of financial loss in the cattle industry. The mean incidence of diarrhea in individual herds of cattle can be as high as 80% (Cornaglia *et al*., 1992; Khan and Khan, 1995; Wright *et al*., 1995); therefore, rates of diarrhea > 50% are not unusual. The mortality rate due to diarrhea varies up to 25% (Khan and Khan, 1995; Buenau *et al*., 2005; Khan *et al*., 2009). Successful treatment of diarrheic calves based primarily on the use of broad-spectrum antibiotics or other antimicrobials along with administration of fluids and electrolytes for the correction of dehydration and acidemia (Roussel and Kasari, 1990; Berchtold, 1999; Senturk, 2003; Buenau *et al*., 2005). Therefore, intravenous administration of isotonic fluids is considered to be the best methods for treating severely dehydrated and acidotic calves with diarrhea (Simmons *et al*., 1985; Roussel and Kasari, 1990; Walker *et al*., 1998; Berchtold, 1999; Senturk, 2003). It is recommended @ 90 mL/kg BW which is a large volume to infuse throughout a 3 to 4 h period to correct hydration status (Roussel and Kasari, 1990; Constable *et al*., 1996; Walker *et al*., 1998; Flores *et al*., 2006; Hasanpour *et al*., 2009). Infusion of required volumes of isotonic fluids is quite difficult in on-farm situations, because of the requirement of more than one catheterization, proper restraint and periodic monitoring (Constable *et al*., 1996; Walker *et al*., 1998). So, a more practical method for IV administration of fluids requiring small volumes would be advantageous.

Hypertonic saline solution (7–7.5% NaCl) has gained much popularity for the last two decades as a small-volume, rapid and effective method for fluid administration in severely dehydrated diarrheic calves (Constable *et al*., 1996;

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Walker *et al*., 1998; Senturk, 2003; Flores *et al*., 2006) and has also been successfully used to resuscitate humans, sheep, horses, dogs and cats with hypovolemia and/or endotoxemia (Velasco *et al*., 1980; Nakayama *et al*., 1984; Constable *et al*., 1991a; Kramer *et al*., 2003; Somell *et al*., 2007). Intravenous administration of HSS causes an initial rapid influx of fluid into the vasculature due to the sudden hypertonic state of plasma in a relatively short time (Constable *et al*., 1991a; Walker *et al.*, 1998; Bleul *et al.*, 2007; Leal *et al*., 2012). Plasma volume expansion is, therefore, achieved with lesser free water administration than with isotonic solutions. Since administration of a hypertonic should lead to recruitment of extravascular fluids into the vascular compartment, HSS seems likely to produce a more rapid response and marked hemodynamic effects than isotonic solutions (Bleul *et al*., 2007; Leal *et al*., 2012) with a small-volume @ 4 mL/kg BW.

Enough information is available on use of HSS in calf diarrhea, however, information on its use in neonatal calf diarrhea associated with *E. coli* is totally nonexistent. So, this study was planned to evaluate the efficacy of HSS as an adjunct to ceftiofur HCl (a third generation cephalosporin) and flunixin meglumine (a NSAID) in neonatal diarrhea associated with *E. coli* in calves and to compare its resuscitative response with that of ISS along with ceftiofur HCl and flunixin meglumine.

## Materials and Methods

### Experimental Animals

The experiment was conducted in accordance with a protocol approved by the Institutional Animal Ethics Committee, University of Agriculture, Faisalabad, Pakistan. The study was conducted on 24 University-owned, colostrum-fed healthy male buffalo calves of 5–7 days old and with a mean weight of 35 ± 4.5 kg. Animals underwent a 7 days acclimatization period. These calves were maintained at Livestock Experimental Station, University of Agriculture, Faisalabad, Pakistan. Animals were housed in individual movable iron pens 30 cm above the ground and with wooden-strip floor. The diet consisted of a milk replacer (Telelac®, ICI Animal Health Division, Pakistan) administered PO through bottles with nipple, bid @ 10 percent of body weight per calf per day.

### Instrumentation

The day prior to induction of neonatal diarrhea, the calves were sedated with xylazine HCl (Xylaz®, Farvet Laboratories, Holland) @ 0.02 mg/kg of body weight, IM, for aseptic placement of intravenous catheters. The hair over the right and left jugular furrows were clipped and the skin was scrubbed surgically for aseptic placement of IV catheters. For infusion of solutions and collection of blood samples, catheter of 18-gauge was placed in right jugular vein, while left jugular vein was catheterized with 7-F thermodilution Swan-Ganz catheter for recording central venous pressure (CVP). Girdle area was shaved and prepared aseptically for cannulation of femoral artery with the help of 22-gauge catheter for the measurement of mean arterial pressure (MAP) and collection of blood samples for determination of partial pressure of arterial oxygen ($PaO_2$). Jugular catheters were flushed with 1 mL and 0.5 mL saline (0.9% NaCl) solution containing 5 U of sodium heparin/mL at the time of infusion of solutions and sampling, respectively. Swan-Ganz and femoral artery's catheters were flushed with heparinized saline solution containing 40 IU heparin/mL before measuring the CVP and MAP, respectively. Xylazine sedation was reversed by administering tolazoline HCl (1 mg/kg) (Tolazin®, Lloyd Laboratories Inc., USA) after catheterization. The calves were placed in movable pens 12 h after instrumentation. Baseline values were then recorded by obtaining blood samples.

### Induction of Diarrhea

An enteropathogenic *E. coli* (antigen K99) isolated from a field case of neonatal calf diarrhea was procured from National Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan. Infection was introduced through oral administration of 2 mL broth culture (*E. coli* count = $10^{10}$ CFU/mL dissolved in 250 mL of normal saline to each calf of both groups (Brooks *et al*., 1996; Zaman, 2001). Diarrhea was induced successfully in all the calves within 6 to 8 h. Twelve h after the onset of diarrhea (10% dehydration, skin tenting time > 3 but < 8 seconds, markedly sunken eyes), calves of both the groups were considered for the treatment phase.

### Experimental Design

At the start of treatment phase, calves were randomly allocated through random number table (Chow and Lin, 2004) to either a control or a test group ($n$=12 calves/group). All fluids used in the treatment phase were warmed to 37°C immediately before administration. Calves in control group (Group A) were infused intravenously with ISS (0.9% NaCl) @ 90 mL/kg BW along with ceftiofur HCl @ 6 mg/kg BW, IM and flunixin meglumine @ 2 mg/kg BW, IM. Calves of group B (test group) were dosed intravenously with HSS (7.5% NaCl) @ 4 mL/kg BW during a 4-min period via jugular vein followed by ISS @ 10 mL/kg BW, in combination with ceftiofur HCl @ 6 mg/kg BW, IM and flunixin meglumine @ 2 mg/kg BW, IM.

In both groups, fluids were infused once at the first day treatment, while ceftiofur HCl and flunixin meglumine were repeated after 24 and 12 h, respectively. Administration of HSS provided 4.9 mmol of Na/mL.

The physical condition of each calf was assessed throughout the study and calves that were unable to stand without assistance were not considered for study. At the completion of the study, milk replacer feeding was reinstated and administration of conventional IV fluid was instituted as needed, determined on the clinical assessment.

**Measurement and Analysis of Samples**

Clinical parameters (survival rate, rectal temperature, respiratory rate and heart rate), hematological profiles (hemoglobin concentration, hematocrit), serum electrolytes (sodium, chloride and potassium ions concentration), blood gas analysis [partial pressure of arterial oxygen ($PaO_2$), venous carbon dioxide ($PvCO_2$), venous blood pH and bicarbonates($HCO_3^-$)] and hemodynamic parameters (mean arterial pressure, central venous pressure) were measured to evaluate the comparative resuscitative effects of HSS and ISS along with ceftiofur HCl and flunixin meglumine in *E. coli* associated neonatal diarrhea.

For hematological analyses, 2 mL of blood was collected into evacuated tubes that contained EDTA. The hemoglobin concentration (Hb conc.) was determined by cyannmethemoglobin method and hematocrit (HCT) values were determined with microhematocrit method as described by Benjamin (1978). For blood gas analysis, 1 mL blood was collected anaerobically in heparinized syringe and the tip was capped (Constable *et al*., 1996). The syringe was placed on ice and processed within an h of collection by automatic gas analyzer (Blood Gas Analyzer, Medica Corporation, Bedford, USA). Values were automatically corrected to rectal temperature. For measurement of electrolytes, 5 mL of blood was collected into evacuated tubes without anticoagulant to harvest serum followed by centrifugation and storage at -20$^o$C until assayed. The serum was then analyzed for sodium, chloride and potassium ions concentration through flame photometer.

All the mentioned parameters were recorded at baseline (before induction of diarrhea), during diarrhea, t=1 h, t=3 h, t=6 h, t=12 h, t=24 h and t=36 h after institution of allotted treatment to the groups.

**Statistical Analyses**

The significance of differences between the treatment groups was analyzed by a student t-test. Differences between points of time within a group were checked by one-way analysis of variance (ANOVA). The significance of differences between groups was tested by Duncan's Multiple Range (DMR) Test. Differences were classified as significant if $P < 0.05$ (Steel and Torrie, 2004).

## Results

A total of 24 colostrum-fed, male calves formed the basis of part of the present study. After adaptation period of 7 days, these calves were induced diarrhea with oral administration of *E. coli* broth culture. After 6 to 8 h, the protocol of diarrhea induction resulted in intense aqueous diarrhea in 100% of the animals. Twelve h after the onset of diarrhea, the calves with 10% dehydration were treated with ISS (group A) and HSS (group B) saline solutions in combination with ceftiofur HCl and flunixin meglumine. Mean values of all parameters observed differed non-significantly in calves of control and test groups prior and during infection.

**Clinical Parameters**

**Survival Rate:** Each group comprised of 12 calves after induction of diarrhea. After treatment, mortality was observed in calves of both groups but it was higher in ISS treated group (group A) than HSS treated (group B). However, statistical difference was non-significant (Table 1).

**Body Temperature:** A significant increase in body temperature was observed during diarrhea in calves of both groups. After treatment, a significant decrease was noted in both groups, however, group B showed significant difference ($P < 0.05$) over group A at t=1 h. Both groups attained basal values within 12 h (Table 2).

**Respiration Rate:** A significant decrease was observed in respiration rate in calves of both groups during infection which increased significantly after administration of specific treatment protocols at t=1 h in both groups followed by significant decrease again. At t=6 h, group A showed significant difference ($P < 0.05$) over group B. After that a rapid increase was observed in group B and it showed a significant difference ($P < 0.05$) over its counterpart at t=24 h (Table 2). Both groups recovered the baseline values within observing period.

**Heart Rate:** Increased heart rate was observed during diarrheic condition in both groups. After institution of treatments, heart rate decreased significantly ($P < 0.05$) in both groups. Both groups followed a sharp decreasing trend toward baseline values (Table 2), however, group A differed significantly ($P < 0.05$) from group B at t=3 h when a transitly increased heart rate was observed in group B followed by significant increase in its values (Table 2).

**Hematological Analyses**

**Hematocrit:** Increased values of HCT were noted during diarrhea. Treatment institution to the calves helped in decreasing the increased values of HCT significantly in both groups. Both groups showed good recovery towards baseline but better recovery rate was observed in calves of group B (Table 2). The baseline values were recovered within 12 h post-treatment in both groups.

**Hemoglobin Concentration:** The protocol to induce diarrhea increased concentration of Hb in neonate calves.

**Table 1:** Survival rate of buffalo calves treated with two different treatment protocols after induction of *E. coli* associated diarrhea

| | Time after treatment (hours) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | 1 | 3 | 6 | 12 | 24 | 36 |
| Group A | 12 | 11 | 11 | 10 | 10 | 09 | 09 |
| Group B | 12 | 12 | 11 | 11 | 11 | 11 | 11 |

Group A (*n=12* calves/group) suffering with diarrhea associated with *E. coli* and response to treatments with IV infused ISS along with ceftiofur and flunixin; Group B (*n=12* calves/group) suffering with diarrhea associated with *E. coli* and response to treatments with IV administered HSS in combination with ceftiofur and flunixin

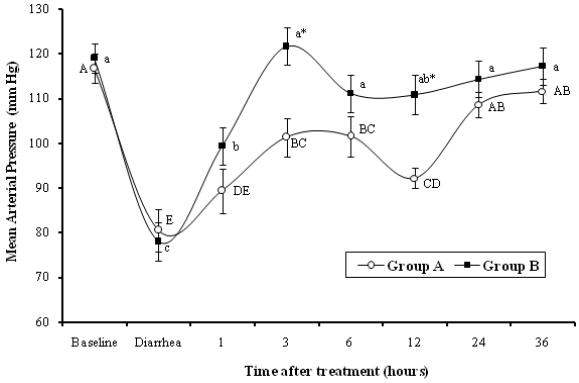


**Fig. 1:** Mean arterial pressure (mm Hg) in buffalo calves (*n=12* animals/group) with induced neonatal diarrhea and response to treatments with IV administered ISS (group A) and HSS (group B) in combination with ceftiofur and flunixin
*Indicates significant difference ($P > 0.05$) between Group A and Group B at that time (h). Means (± SE) sharing similar letters within a group are statistically non-significant

After administration of treatment to groups A and B, values of Hb conc. decreased significantly ($P < 0.05$). Both treatment protocols showed similar trend in recovering the Hb conc. toward normal (Table 2). The baseline values were recovered within experimentally time in both groups.

### Hemodynamic Parameters

**Mean arterial pressure:** During diarrhea, MAP decreased significantly ($P < 0.05$) in both groups. Within first h after treatment (t=1 h), it increased in both groups but group B showed significant increase ($P < 0.05$) and baseline values were achieved at t=3 h after infusion of HSS and showed significant difference ($P < 0.05$) over group A, while MAP was near to normal in group A at t=36 h (Fig. 1).

**Central Venous Pressure:** It decreased significantly in all the calves with induced diarrhea. After institution of treatment, a significant increase was observed in both groups but group B which was treated with HSS recovered the basal values at t=3 h and showed significant difference ($P < 0.05$) over group A throughout the study except at t=6 h, at which it showed significant fall in CVP (Fig. 2). Administration of ISS to group A was unable to recover the CVP to baseline within experimental period.

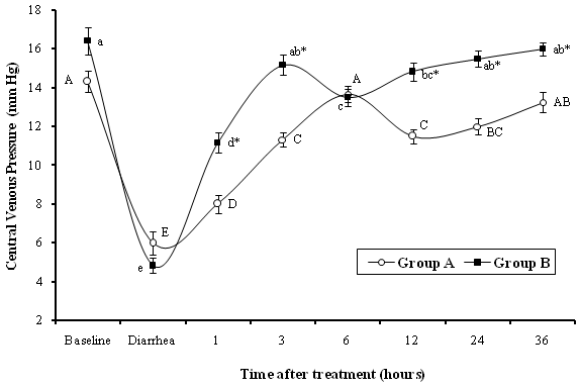


**Fig 2:** Central venous pressure (mm Hg) in buffalo calves (*n=12* animals/group) with induced neonatal diarrhea and response to treatments with IV administered ISS (group A) and HSS (group B) in combination with ceftiofur and flunixin. *indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours). Means (± SE) sharing similar letters within a group are statistically non-significant

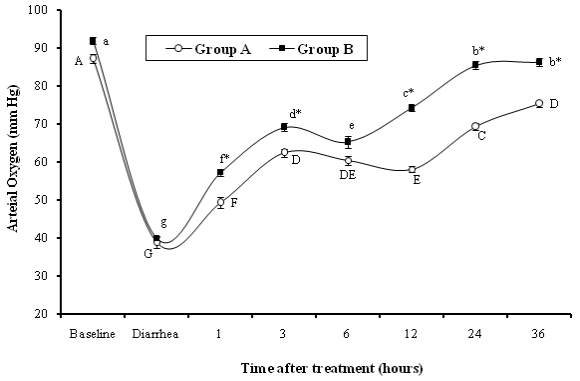


**Fig. 3:** Partial pressure of arterial oxygen (mm Hg) in buffalo calves (*n=12* animals/group) with induced neonatal diarrhea and response to treatments with IV administered ISS (group A) and HSS (group B) in combination with ceftiofur and flunixin
*Indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours). Means (± SE) sharing similar letters within a group are statistically non-significant

### Blood Gas Analyses

**Arterial Oxygen:** A significant decrease was observed in arterial pressure of oxygen ($PaO_2$) during infection which increased significantly in both groups after treatment. However, group B showed better recovery toward basal values and showed significant difference ($P < 0.05$) over group A at each observational time throughout the experimental period (Fig. 3).

**Venous Carbon Dioxide:** A significant increase was noted in $PvCO_2$ during diarrhea. After institution of treatment,

**Table 2:** Results of serum electrolytes, hematology and clinical parameters for samples obtained from neonate calves induced with diarrhea before and after treatment in two groups

| Variables | Groups | Time after treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Baseline | Diarrhea | t=1 h | t=3 h | t=6 h | t=12 h | t= 24 h | t=36 h | |
| Sodium | Group A | 135.17±11.72$^{A}$ | 123.58±12.65$^{B}$ | 124.83±13.33$^{B}$ | 129.42±13.51$^{AB}$ | 132±14.17$^{AB}$ | 129.17±14.89$^{AB}$ | 135.33±13.45$^{A}$ | 135.25±10.81$^{A}$ |
| (mmol/L) | Group B | 133.25±14$^{bc}$ | 124.42±13.91$^{c}$ | 133.33±12.29$^{bc}$ | 143.5±10.75$^{a*}$ | 136.08±11.86$^{ab}$ | 139.17±11.85$^{ab*}$ | 137.5±10.66$^{ab}$ | 133.33±10.70$^{bc}$ |
| Chloride | Group A | 97.5±8.98$^{A}$ | 79.58±10.77$^{D}$ | 84.75±10.08$^{CD}$ | 91.17±8.92$^{ABC}$ | 85.33±8.05$^{BCD}$ | 93.08±9.13$^{AB}$ | 96.33±10.38$^{A}$ | 96.75±11.22$^{A}$ |
| (mmol/L) | Group B | 101.92±9.74$^{a}$ | 81.58±15.23$^{c}$ | 91.25±15.46$^{bc}$ | 104.67±13.5$^{a*}$ | 97.08±10.91$^{ab*}$ | 96.83±11.02$^{ab}$ | 100.75±11.11$^{ab}$ | 101.92±11.97$^{a}$ |
| Potassium | Group A | 4.58±1.29$^{A}$ | 3.34±1.44$^{A}$ | 3.5±1.07$^{A}$ | 3.8±1.05$^{A*}$ | 3.37±1.1$^{A}$ | 3.54±1.22$^{A}$ | 4±1.03$^{A}$ | 4.44±1.01$^{A}$ |
| (mmol/L) | Group B | 4.72±1.17$^{a}$ | 3.69±1.18$^{ab}$ | 3.37±1.06$^{b}$ | 3.08±1.15$^{b}$ | 3.53±1.34$^{b}$ | 3.8±1.42$^{ab}$ | 3.94±1.51$^{ab}$ | 4.58±1.22$^{a}$ |
| Hematocrit | Group A | 34±4.43$^{B}$ | 40.17±4.02$^{A}$ | 37.08±4.4$^{AB}$ | 33.42±5.62$^{B}$ | 35.33±5.91$^{B}$ | 33.58±4.48$^{B}$ | 34±4.39$^{B}$ | 34.42±4.89$^{B}$ |
| (%) | Group B | 32.75±5.88$^{b}$ | 39.42±6.92$^{a}$ | 34.5±6.35$^{b}$ | 35.25±5.58$^{b}$ | 35.58±4.5$^{b}$ | 33.17±5.64$^{b}$ | 31.75±4.8$^{b}$ | 32.33±4.56$^{b}$ |
| Hb conc | Group A | 11.67±2.39$^{A}$ | 15.25±3.22$^{A}$ | 12.67±4.03$^{A}$ | 11.50±3$^{A}$ | 11.83±3.33$^{A}$ | 12.75±3.36$^{A}$ | 11.67±2.9$^{A}$ | 11.83±3.71$^{A}$ |
| (g/dL) | Group B | 11.83±2.59$^{b}$ | 16±2.04$^{a}$ | 12.08±2.11$^{b}$ | 11.92±2.39$^{b}$ | 13.17±2.21$^{b}$ | 11.58±1.44$^{b}$ | 11.75±1.36$^{b}$ | 11.75±1.71$^{b}$ |
| Body Temp | Group A | 38.33±0.12$^{DE}$ | 39.27±0.15$^{A}$ | 38.90±0.14$^{B}$ | 38.44±0.12$^{C}$ | 38.36±0.12$^{CD*}$ | 38.23±0.12$^{E}$ | 38.35±0.12$^{CD}$ | 38.37±0.12$^{D}$ |
| ($^{o}$C) | Group B | 38.22±0.16$^{c}$ | 39.35±0.26$^{a}$ | 38.67±0.23$^{b*}$ | 38.37±0.14$^{c}$ | 38.66±0.19$^{b}$ | 38.24±0.14$^{c}$ | 38.27±0.17$^{c}$ | 38.28±0.15$^{c}$ |
| Heart rate | Group A | 81±5.94$^{E}$ | 105.67±3.98$^{A}$ | 95.33±4.46$^{B}$ | 85.67±6.02$^{D*}$ | 90±7.53$^{C}$ | 82.33±4.66$^{DE}$ | 81±4.86$^{E}$ | 83.33±2.87$^{DE}$ |
| (beats/min) | Group B | 86±6.03$^{cd}$ | 110.67±4.29$^{a}$ | 90.33±3.98$^{c}$ | 97±5.43$^{c}$ | 89.33±6.68$^{c}$ | 83.33±6.79$^{d}$ | 86.33±5.25$^{cd}$ | 86.33±3.98$^{cd}$ |
| Resp. rate | Group A | 30.67±3.55$^{A}$ | 21.33±2.61$^{C}$ | 28.67±2.87$^{B}$ | 25±3.46$^{C}$ | 30.33±3.98$^{A*}$ | 29.33±3.94$^{A}$ | 30.33±4.66$^{A}$ | 31.33±3.34$^{A}$ |
| (breadths/min) | Group B | 32.33±4.66$^{ab}$ | 20.67±4.12$^{d}$ | 30.33±4.33$^{b}$ | 26.67±3.55$^{c}$ | 24.33±2.67$^{c}$ | 30.67±2.61$^{ab}$ | 33.33±2.61$^{a*}$ | 31.17±3.56$^{ab}$ |

Group A (*n=12* calves/group) suffering with diarrhea associated with *E. coli* and response to treatments with IV infused ISS along with ceftiofur and flunixin; Group B (n=12 calves/group) suffering with diarrhea associated with *E. coli* and response to treatments with IV administered HSS in combination with ceftiofur and flunixin

* indicates significant difference ($P > 0.05$) between Group A and Group B at that time (h). Means (± SE) sharing similar letters within a group are statistically non-significant

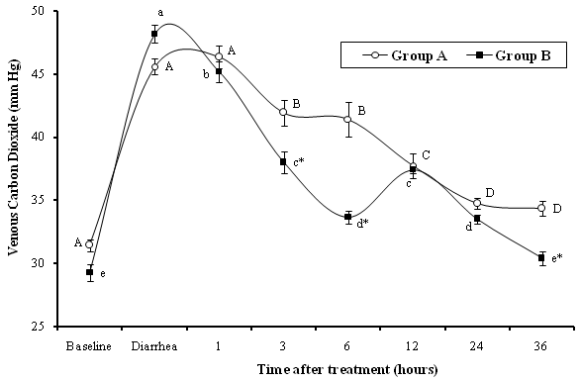


**Fig. 4:** Partial pressure of venous carbon dioxide (mm Hg) in buffalo calves (*n=12* animals/group) with induced neonatal diarrhea and response to treatments with IV administered ISS (group A) and HSS (group B) in combination with ceftiofur and flunixin

*indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours). Means (± SE) sharing similar letters within a group are statistically non-significant

group B showed significant decrease in $PvCO_2$ at t=1 h, while no change was observed in group A. At t=3 h, group A showed significant ($P < 0.05$) decrease in $CO_2$ pressure but it was continuous in group B which showed significant difference ($P < 0.05$) over group A except at t=12 h and at t=24 h, where, a transit increase in values were observed. Group A showed decreasing trend in $PvCO_2$ toward baseline but failed to achieve it within study period (Fig. 4), while HSS administration to group B recovered the baseline values successfully.

**Blood pH:** During diarrhea, significant decrease ($P<0.05$) in blood pH values was observed in both groups. After treatment, both groups showed a slow increasing trend toward baseline and differed non-significantly from each other. Both groups were unable to achieve the baseline values of blood pH within study period, albeit these values were near to baseline at t=36 h (Fig. 5).

**Bicarbonates:** During diarrhea, a significant decrease ($P<0.05$) was observed in values of $HCO_3^-$ in both groups. After treatment, $HCO_3^-$ values increased in group A and showed significant difference ($P < 0.05$) over group B at t=1 h. Group A showed continuous increasing trend toward baseline, while group B showed fluctuating trend of increasing and decreasing at different intervals (Fig. 6). At t=36 h, both groups recovered the $HCO_3^-$ values near to baseline.

## Serum Electrolytes

**Sodium Ions Concentration:** Hyponatremia was observed in calves subjected to the protocol of induced diarrhea. A significant increase ($P < 0.05$) in the sodium concentration was observed at t= 3 h after infusion of HSS to calves of group B. Contrarily, a non-significant difference was noted in group A treated with ISS (Table 2). The $Na^+$ ions concentration decreased after t=3 h rapidly in group B and matched with basal values within experimental period and it also showed significant difference ($P < 0.05$) to group A at t=3 h and t=12 h. On the other hand, group A attained basal values at t=24 h (Table 2).

**Chloride Ions Concentration:** During diarrhea, there was hypochloremia in the calves of all groups subjected to protocol of induction of diarrhea. In HSS treated group (group B), in contrast to its counterpart group (group A), $Cl^-$ ions concentration increased significantly ($P < 0.05$) after treatment and showed significant difference at t=3 h by

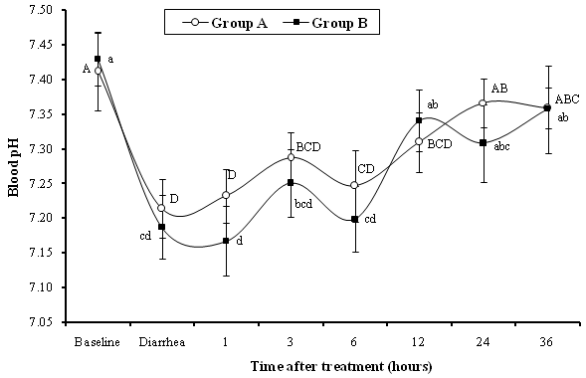


**Fig. 5:** Blood pH in buffalo calves (*n=12* animals/group) with induced neonatal diarrhea and response to treatments with IV administered ISS (group A) and HSS (group B) saline solutions in combination with ceftiofur and flunixin
*indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours). Means (± SE) sharing similar letters within a group are statistically non-significant

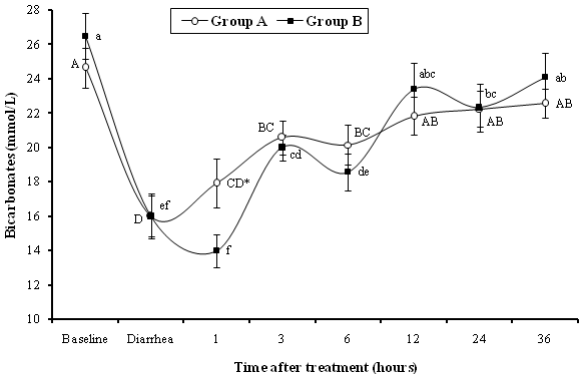


**Fig. 6:** Bicarbonates (mmol/L) in buffalo calves (*n=12* animals/group) with induced neonatal diarrhea and response to treatments with IV administered ISS (group A) and HSS (group B) in combination with ceftiofur and flunixin
*indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours). Means (± SE) sharing similar letters within a group are statistically non-significant

attaining peak value. At t=6 h, $Cl^-$ ions concentration decreased ($P < 0.05$) and attained the baseline value (Table 2). The concentration of $Cl^-$ ions in calves treated with ISS (group A) increased and reached the basal value at t=24 h (Table 2).

**Potassium Ions Concentration:** Induction of diarrhea affected a significant decrease in serum concentration of $K^+$ ions compared to their basal values. Concentrations of $K^+$ ions in calves treated with HSS and ISS presented different results during study period (Table 2). In group B treated with HSS, $K^+$ ions concentration first decreased followed by a rapid increase and matched with baseline ($P > 0.05$) at t=36 h. In group A treated with ISS, $K^+$ ions concentration increased and significantly differed ($P < 0.05$) from group B at t=3 h. However, basal values were recovered at t=36 h (Table 2).

## Discussion

The present study was conducted to evaluate the relative efficacy of two crystalloids (ISS and HSS) in combination with parentral administration of an antibiotic (ceftiofur HCl) and a NSAID (flunixin meglumine) in calves in which diarrhea had been induced by the oral administration of *E. coli* (K99). The protocol to induce neonatal diarrhea promoted severe diarrhea in 100% of the calves. A moderate dehydration around 10%, hyponatremia, hypochloremia, hypokalemia, decreased arterial oxygen pressure, blood pH, bicarbonates and increased body temperature, heart rate, partial pressure of carbon dioxide were recorded during induced neonatal diarrhea.

Dehydration in calves with neonatal diarrhea is accompanied by a decrease in extracellular fluid volume resulting in decreased plasma volume (Walker *et al*., 1998). The aim for the treatment of severely dehydrated calves with neonatal diarrhea is to restore plasma volume rapidly, thereby improving cardiac output, increasing mean arterial pressure and oxygen delivery and correcting acid-base and electrolyte imbalance.

The protocol for induction of neonatal diarrhea with *E. coli* (K99) caused mortality in calves of both groups but decreased mortality was observed in group B which can be attributed to the use of HSS in this treatment group. In the present study, increased heart rate was observed in dehydrated diarrheic calves. These results have already been envisaged in previous studies that decrease in plasma volume stimulates sympathetic nervous system which increases the heart rate through peripheral vasoconstriction. It also stimulates rennin-angiotensin-aldosterone system to maintain mean arterial pressure, heart balance and organ perfusion (Flores *et al*., 2006; Bleul *et al*., 2007). In calves with diarrhea, heart rate decreased toward baseline after administration of ISS and HSS to groups A and B, respectively. An immediate expansion in plasma volume due to increased serum osmolarity is the contributing factor for activation of cardiovascular reflex mechanisms (Bertone *et al*., 1990; Constable *et al*., 1995; Bleul *et al*., 2007). These effects were, however, transient; lasting only for one h and then heart rate dropped and became normal rapidly.

The results of the present study indicated increased hematocrit and Hb conc. in dehydrated diarrheic calves. Similar results have also been reported in previous studies as increase in HCT and Hb conc. is the cause of decreased plasma volume (Constable *et al*., 1991b; Senturk, 2003). In the studies conducted by Constable *et al*. (1991b) and Senturk (2003) wherein intervention with a fixed volume of HSS with or without dextran was undertaken after endotoxin infusion, an immediate response to fluid treatment (with a decrease in HCT and Hb conc.) was found indicating expansion in intravascular volume. We also found a rapid decrease in values of HCT and Hb conc. in this study which indicates that fluid resuscitation with HSS was effective for plasma volume expansion.

Hypertonic saline solution caused immediate and significant increase in MAP and CVP values which had fallen in calves as a consequence of neonatal diarrhea. This is most likely a volume resuscitation effect associated with increased preload and inherent positive inotropic effect of HSS (Somell *et al*., 2007). The other factor involved is the rapid plasma volume expansion, which occurs within 1 h of the infusion because of the immediate increase in serum osmolarity and serum sodium concentration, promoting fluid circumvent from intracellular compartment and gastrointestinal tract (Constable *et al*., 1991b; Rocha e Silva, 2005). Some previous studies e.g. Constable *et al*. (1996) and Walker *et al*. (1998) have recommended the use of 6% dextran-70 in association with HSS to maintain plasma expansion. However, our results point to the potential of HSS to cause plasma expansion without dextran. These findings of our study are also supported by the results reported by Flores *et al*. (2006).

Blood gas analyses are frequently used to determine the imbalance of acid/base status and it also helps to develop effective treatment plan to resuscitate the patient from acidemia. In the present study, decreased arterial oxygen pressure ($PaO_2$), increased venous carbon dioxide pressure ($PvCO_2$), decreased blood pH and bicarbonates were noted in calves suffering from neonatal diarrhea. Decreased $PaO_2$ indicated that animals were suffering from hypoxemia. It could be due to the endothelial cell edema in the endothelium of postcapillary venules which cause obstruction in the blood flow and reduction in oxygen transport (Oliveira *et al*., 2002). It is also hypothesized that HSS induces endothelial cell shrinkage resulting in improved tissue perfusion and oxygen delivery (Oliveira *et al*., 2002; Koch and Kaske, 2008; Leal *et al*., 2012). Infusion of HSS to the calves of group B also decreased the pressure of $CO_2$ resulting in increased blood pH and bicarbonates which helped in recovering the calves from acidosis. These results are supported by the findings of several previous studies (Constable *et al*., 1991b; Senturk, 2003; Somell *et al*., 2007; Koch and Kaske, 2008; Hasanpour *et al*., 2009; Leal *et al*., 2012).

Hyponatremia has previously been reported in *E. coli* associated diarrhea in cow calves (Constable *et al*., 1996; Berchtold, 1999; Flores *et al*., 2006). In contrast to normal saline, administration of HSS immediately increased serum sodium concentration in buffalo calves but it remained below the value of hypernatremia (reference value = 160 mmol/L; Constable *et al*., 1996). No evidence of severe hypernatremia has been reported after using HSS @ 4 mL/kg BW (Rocha e Silva, 2005; Koch and Kaske, 2008; Leal *et al*., 2012). In our study, the highest serum sodium after the initial fluid administration was 143.5 ± 10.75 mmol/L. However, HSS is contradicted in chronic symptomatic or asymptomatic hyponatremia as it causes neurological complications (Dibartola, 2000). In our study, no abnormality in the behaviour and attitude of the buffalo calves was observed after infusion of HSS.

## Conclusion

On the basis of findings of this study, it was concluded that HSS can be safely administered to the buffalo calves suffering with neonatal diarrhea associated with *E. coli*. It offsets deleterious hemodynamic effects of hypovolemia, improves oxygenation, corrects metabolic acidosis and leads to increased survival rate in buffalo calves suffering from *E. coli* induced diarrhea. The small volume of HSS seems a more practical and economical alternative to the use of large volumes of isotonic saline solution for use in field settings. In view of the preliminary nature of the present study, additional work on use of HSS in neonatal buffalo calf diarrhea and acid base status is clearly warranted. As also is the need to test the efficacy of HSS in the treatment of spontaneously occurring diarrhea in buffalo calves.

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