Evaluation of Hypertonic Saline Solution in the Treatment of Haemorrhagic Septicaemia, Calf Scour and Other Clinical Conditions of Livestock Characterized by Hypovolaemia / Endotoxaemia

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DVM, M.Sc (Hons.)

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Degree of

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FACULTY OF VETERINARY SCIENCE
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2010
To

The Controller of Examinations,

University of Agriculture,

Faisalabad.

“We, the Supervisory Committee, certify that the contents and form of thesis submitted by Mr. Muhammad Arif Zafar, Regd. No. 97-ag-1537, have been found satisfactory and recommend that it be processed for evaluation by the External Examiner(s) for the award of doctoral degree”

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MUHAMMAD ARIF ZAFAR
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This study was conducted for the evaluation of hypertonic saline solution in haemorrhagic septicaemia (HS) in buffaloes, calf scour in buffalo calves and other clinical conditions of livestock characterized by hypovolaemia and/or endotoxaemia including spontaneous cases of calf scour, dehydrated diarrhoeic goats and buffaloes. For this purpose, 50 buffaloes were selected from the field suffering from HS. The disease was diagnosed on the basis of clinical signs. Then theses animals were randomly divided into two equal groups (A and B). Buffaloes of group A were treated with the conventional treatment already in vogue i.e. ceftiofur HCl and flunixin meglumine @ 6 mg/kg and 2 mg/kg BW, IM and IV, respectively. Group B was treated with intravenous infusion of hypertonic saline solution (HSS) @ 4 mL/kg BW followed by isotonic saline solution @ 10 mL/kg BW along with ceftiofur HCl and flunixin meglumine. For evaluation of HSS in calf scour, neonatal diarrhoea in buffalo calves (n=24) was induced through oral administration of 2 mL broth culture of having enteropathogenic E. coli count of 10^{10} CFU. To evaluate the efficacy of HSS in clinical conditions of livestock, spontaneous cases of calf scour (n=24), dehydrated diarrhoeic goats (n=24) and buffaloes (n=24) were studied. In all these conditions, the animals were randomly divided into two equal groups viz. A and B (n=12 each). Group A was treated with isotonic (90 mL/kg BW) and group B with hypertonic (4 mL/kg BW) saline solutions along with ceftiofur HCl and flunixin meglumine (6 and 2 mg/kg BW, respectively). The efficacy of treatment was evaluated on the basis of clinical parameters, haematological analysis, haemodynamic parameters, blood gas analysis, serum electrolytes and serum biochemical profiles. These all evaluation parameters were recorded at baseline (during disease), t=1, t=3, t=6, t=12, t=24 and t=36 hours after treatment. However, for induced calf scour, the additional recording time point was before induction of diarrhoea which acted as baseline; other recording time points being the same as for other conditions studied. Hypertonic saline infusion to the buffaloes of group B suffering from HS showed significantly higher survival rate of 80% and differed significantly (P < 0.05) from group A in which survival rate was 52%. Group B significantly (P < 0.05) improved heart rate, mean arterial pressure, central venous pressure, haematocrit and haemoglobin concentration, partial pressure of arterial oxygen, blood pH and total serum protein. Hypertonic infusion increased serum sodium and
chloride ions concentration but the changes were not of sufficient magnitude to be of risk to the buffaloes. Hypertonic saline solution (group B) resuscitated buffalo calves from neonatal diarrhoea (either induced or spontaneous) more rapidly and effectively than isotonic saline solution (group A) and showed significant \( P < 0.05 \) improvement in all the parameters studied. The resuscitation of diarrhoeic dehydrated goats \((n=24)\) and buffaloes \((n=24)\) through administering hypertonic saline solution was evaluated. Both treatment protocols helped in recovering the normal values of all the parameters studied within experimental period. But hypertonic saline solution showed significant differences \( P < 0.05 \) over group A in heart rate, mean arterial pressure, central venous pressure, partial pressure of arterial oxygen, blood pH and bicarbonates in diarrhoeic goats. In diarrhoeic buffaloes, HSS infusion only showed significant difference \( P < 0.05 \) over group A in partial pressure of venous oxygen, while other parameters were recovered to normal without any statistical difference. On the basis of findings of this study, it was concluded that hypertonic saline solution can be safely administered to the buffaloes suffering from haemorrhagic septicaemia and buffalo calves with neonatal diarrhoea. It offset deleterious haemodynamic effects of endotoxins, thus ameliorates the septic shock more effectively than does antibiotic therapy alone in HS. In addition to rapid and effective, intravenous administration of a small volume of HSS provides a practical and economical method to resuscitate dehydrated calves with neonatal diarrhoea, diarrhoeic goats and buffaloes, thus make it suitable for field use.
CHAPTER 1

INTRODUCTION

In terms of morbidity as well as mortality, haemorrhagic septicaemia (HS) is one of the most important diseases of buffalo and cattle in Pakistan (Ajmal et al., 1988; Raza et al., 2000; Ashraf et al., 2009). There is ample evidence that buffaloes (Bubalus bubalis) are three times more susceptible to this disease than cattle (De Alwis, 1992; Benkirane and De Alwis, 2002). In Pakistan, HS causes huge economic losses each year and is considered as the most fatal disease. Accounting for annual losses of Rs. 2.17 billions, this disease was ranked number one among the economically important livestock diseases in Punjab province of Pakistan. It was further estimated that a 50% reduction of pecuniary losses associated with this disease through vaccination would be sufficient to reverse the growing deficit between human population growth and livestock production (Anonymous, 1996).

Haemorrhagic septicaemia is associated with a gram-negative bacterium named Pasteurella multocida serotype B:2. This organism produces endotoxins which are responsible for all manifestations of the disease (De Alwis, 1992; Horadagoda et al., 2001). It is now well recognized that endotoxins play a fundamental role in the development of septicaemic condition of the animal in HS (Horadagoda et al., 2001). These endotoxins are released in response to either body defence system or antibiotics. When monocytes or macrophages sense the presence of these endotoxins, they produce inflammatory mediators including tumour necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6) (Dinarello, 1996; Horadagoda et al., 2001; Gouvy et al., 2005), which trigger a systemic inflammatory response. In severe sepsis, these inflammatory mediators are present in high quantities and affect profoundly on the central circulatory system leading to myocardial depression, pronounced vasodilatation and transudation of fluid from the vascular space into tissues which result in hypovolaemia. Along with widespread microcirculatory disturbances, volume depletion contributes to hypoperfusion, which disturbs the end-organ functions leading to multiple
organ dysfunctions (MOD), and culminates in death (Somell et al., 2005; Somell et al., 2007). Treating hypovolaemia is a central tenet of early management of severe sepsis (Dellinger et al., 2004).

Recent studies have supported the concept that an early restitution of the circulation is of great importance to raise the intravascular volume, improve tissue perfusion and oxygenation, prevent organ failure, maintain cardiac output and blood pressure and ultimately increase the survival rate (Rivers et al., 2001; Somell et al., 2005; Durairaj and Schmidt, 2008). It is, therefore, of great importance to increase blood pressure and maintain adequate cardiac output through restoration of intravascular volume with the help of isotonic fluids administration. In the field, treatment of HS fails partly because affected animals are not given fluid therapy as a single animal requires a large volume of isotonic fluids (about 40-50 litres). Although, fluid therapy with this large volume may be successful, administration of such a considerable volume is not only economically unachievable but also needs labour, more than one catheterization and is time consuming owing to these difficulties, it is often impractical. Thus, effective therapeutic regimens are desperately needed. Although routine vaccination against HS is practiced in the field, many animals remain unvaccinated and they are likely to contract the disease. Even among vaccinated animals, many instances of immunity breakdown have been observed and outbreaks of HS are common due to vaccination failure.

Non-steroidal anti-inflammatory drugs (NSAIDs) are potent inhibitors of arachidonic acid metabolites that can be used to subside the inflammation (Odensvik et al., 1989). Flunixin meglumine, one of the important members of NSAIDs that belongs to propionic acid class, is believed to be a mainstay in the treatment of endotoxemia. Its use has been shown to diminish the detrimental effects of endotoxins by inhibiting cyclooxygenase, which catalyses the oxygenation process of fatty acids, thus significantly decrease the synthesis of prostanoids, most notably thromboxane A$_2$ and prostaglandin F$_{2a}$ (Odensvik et al., 1989; Elmas et al., 2006; Yazar et al., 2007). It also has been reported to improve haemodynamic changes induced by endotoxins specifically decreased mean arterial blood pressure, peripheral vasodilation and pulmonary hypertension (Templeton et al., 1987; Baskett et al., 1997).
Calf scour of neonatal calves caused by *Escherichia coli* and other gut associated microorganisms is another serious welfare problem and considered as an important cause of economic losses. It remains an important and major cause of illness and death in buffalo calves in Pakistan. Mortality in buffalo calves due to diarrhoea varies from 23.7% to 63.0% (Khan and Khan, 1996; Khan *et al*., 2009). Successful treatment of diarrhoeic calves depends primarily on the administration of fluids and electrolytes for the correction of dehydration and acidemia using electrolyte solutions (Barragry, 1994; Radostits *et al*., 2007; Senturk, 2003). Orally administered fluids have advantages over those administered through other routes in the sense of ease, speed of delivery and low cost (Simmons *et al*., 1985). Nonetheless, this route has minimal benefit if animal has poor gastrointestinal absorption, impaired circulation due to severe dehydration and/or hypovolaemia. Therefore, intravenous administration of fluids is considered to be the one of the best methods for treating severely dehydrated and acidotic calves with diarrhoea (Simmons *et al*., 1985; Walker *et al*., 1998). However, infusion of larger volumes of isotonic fluids is much difficult to accomplish in on-farm situations, because of the requirement of more than one catheterization, proper restraint and periodic monitoring (Constable *et al*., 1996). A rapid and effective method for fluid administration in severely dehydrated calves would be beneficial. To this end, there is a need for an alternative fluid that should be a small-volume resuscitative, inexpensive, obviates the need for long time catheterization, lesser labour intensive and time consuming. Most of all, it should be easily applicable in the field.

Hypertonic saline (7-7.5% NaCl) solution (HSS) has been successfully used to resuscitate humans, sheep, horses, dogs, cats and calves with hypovolaemia and endotoxemia (Velasco *et al*., 1980; Nakayama *et al*., 1984; Kramer *et al*., 1986; Constable *et al*., 1991a; Constable *et al*., 1996). Administration of HSS intravenously causes an initial rapid fluid influx into the vasculature due to the sudden hypertonic state of plasma in a relatively short time (Constable *et al*., 1991a; Tyler *et al*., 1994; Walker *et al*., 1998). Plasma volume expansion is, therefore, achieved with less free water administration than with isotonic plasma expanders. Since administration of a hypertonic should lead to recruitment of extravascular fluids into the vascular compartment, HSS
seem likely to produce a more rapid response and marked haemodynamic effects than isotonic solutions (Cambier et al., 1997).

Information on the use of hypertonic saline solution in the therapy of HS is totally nonexistent, whereas, scanty information is available on its use in calf diarrhoea. So, this study was planned to evaluate the efficacy of hypertonic saline solution (HSS) in:

I. Haemorrhagic septicaemia in buffaloes in combination with ceftiofur HCl (an antibiotic) and flunixin meglumine (a non-steroidal anti-inflammatory drug; NSAID).
II. Calf scour in buffalo calves along with ceftiofur HCl and flunixin meglumine, and
III. Other clinical conditions characterized by hypovolaemia and/or endotoxaemia i.e. spontaneous cases of neonatal diarrhoea in buffalo calves, diarrhoeic dehydrated goats and diarrhoeic buffaloes.
CHAPTER 2

REVIEW OF LITERATURE

2.1. Haemorrhagic Septicaemia

History of haemorrhagic septicaemia starts from 1878 when scientists of Barbone noticed a very contagious disease among cattle. They named it as Barbone disease. Haemorrhagic septicaemia (HS) has a wide distribution particularly in tropical countries in Africa and Asia as one of the most fatal diseases of livestock impacting hugely on economy of the country by killing the major livestock (Benkirane and De Alwis, 2002). Likewise, HS occurs as an alarming and devastating disease in cattle and buffaloes in Pakistan and endangers the economic returns of animal production. Accounting for annual losses of Rs. 2.17 billions in Punjab only, this disease was ranked number one among the economically important livestock diseases in Pakistan (Anonymous, 1996). Haemorrhagic septicaemia is associated with *Pasteurella multocida* serotype B:2 (Carter-Heddleston classifications) (Carter and De Alwis, 1989; De Alwis, 1992). It is a gram negative bacterium that produces endotoxins. All manifestations of the disease are due to endotoxins (De Alwis, 1992; Horadagoda et al., 2001). These endotoxins lead to endotoxaemia/septicaemia via triggering a series of mediators including cytokines i.e. tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL1β), interleukin-2 (IL-2) and interleukin-6 (IL-6). Tumour necrosis factor-α has been shown able to reproduce most of the toxic effects induced by endotoxins (Beutler and Kruys, 1995), which are the main cause of clinical signs, pathological changes and death (Horadagoda et al., 2001). In severe sepsis, vasodilation, transudation of fluid from the vascular space into tissues, reduced oral intake, and heightened insensible loss combine to produce hypovolaemia. Along with ventricular dysfunction, arteriolar dilation, and vascular obstruction, volume depletion contributes to impaired global perfusion, threatening the function of critical organs. Treating hypovolaemia is a central tenet of early management of severe sepsis (Dellinger et al., 2004): fluid should be administered to elevate intravascular volume,
increase tissue perfusion, stave off organ failure, and enhance survival (Durairaj and Schmidt, 2008).

Formerly, haemorrhagic septicaemia was treated with antibacterial that have bacteriostatic as well bactericidal activities. Sulphadimidine alone (Manna, 1952) or in combination with crystalline penicillin G (Illahi and Afzal, 1965) was successfully used to treat this fatal disease. Other than these, penicillin G was also used in combination with streptomycin sulphate and was compared with oxytetracycline. Recovery rate was about 73% in the animals that received combination (Ansari, 1968). In 1980, an outbreak of HS was controlled by the administration of sulphamethazine (=sulphadimidine) and buffalo calves were saved successfully from the outbreak at one of the Government livestock farm in Pakistan (Livestock Production Research Institute, Behadur Nagar-Pakistan). No mortality was recorded after the treatment (Kazimi and Haq, 1981). Efficacies of neomycin and oxytetracycline separately and in combination were also investigated and it was concluded that the combination of drugs was more effective than both drug alone and it improved weight gain and feed conversion in artificial induction of salmonellosis and pasteurellosis in swine (Bentley, 1983). The same scientist and his colleague compared efficacy of other drugs i.e. sulbactam-ampicillin and penicillin-dihydrostreptomycin in the treatment of pneumonic pasteurellosis in calves. Calves treated with penicillin-dihydrostreptomycin responded poorly, whereas those treated with sulbactam-ampicillin responded promptly and significant differences between two treatments were observed (Bentley and Cummins, 1983). Long-acting oxytetracycline was also evaluated in artificially rhinitis infection with Pasteurella multocida in swine at the dose rate of 20 mg/kg four times at five day intervals and it was observed that sustained treatment with long-acting oxytetracycline may be effective in control of atrophic rhinitis caused by Pasteurella multocida (Gois et al., 1983). In another study, long-acting oxytetracycline was compared with short acting oxytetracycline for the treatment of pneumonic pasteurellosis in cattle. There was no significant difference found between treatments in terms of recovery rates (Breeze and Magonigle, 1979). Jindal and his colleagues (1996) reported HS outbreaks among buffaloes in India and treated with sulfamethazine and tetracycline along with analgesic and antihistaminic in usual doses. The recovery rate was 61.1% and 14.3%, respectively, proving that tetracycline has only
a minimal role in HS. In the last decade, Fluoroquinolones were also evaluated in pasteurellosis in different susceptible species of the animals. In 1991, Giles with his colleagues evaluated the efficacy of Danofloxacin (a fluoroquinolone) in the treatment of acute bacterial pneumonia associated with Pasteurella multocida and Pasteurella haemolytica in housed beef cattle and compared its clinical response with those treated with oxytetracycline. Cattle were treated for 3 or 5 days (depending on clinical response) with both allocated treatments. These workers observed no difference between recovery rates, however, in comparison with oxytetracycline; danofloxacin therapy was characterized by significantly fewer treatment days, a higher response rate, significantly better reduction of pyrexia and fewer cattle requiring re-treatment. Enrofloxacin has also shown good efficacy against pasteurellosis associated with Pasteurella multocida in rabbits (Broome and Brooks, 1992). The rabbits showed culture negative after 3-7 days after initiation of oral treatment with enrofloxacin. Overwhelmed by the numerous field reports of unsuccessful treatments of HS using conventional antibiotics, and also prompted by excellent in vitro susceptible results, Muhammad (1994) tried a quinolone antibiotic in the treatment of this disease. Eight cases of HS were successfully treated by giving 8-12 ml of enrofloxacin. Although this preparation is indicated for administration through drinking water in poultry, the absolute clarity of the solution tempted this investigator to make an off-label (i.e. parenteral) use in buffaloes. The percent recovery using this product was not stated by this worker. For that reason, Raza et al. (2000) investigated the injectable of Norfloxacin along with Diclofenic sodium (NSAID) and toxin neutralizer circulatory-stimulant (Novacoc-Forte®; Richter Pharma., Austria). These scientists concluded from their studies that use of quinolone (Norfloxacin) plus non-steroidal anti-inflammatory drug (Diclofenic sodium) with or without toxin neutralizer circulatory-stimulant were more effective than conventional line of treatment. Ceftiofur is a third generation veterinary originated Cephalosporin had also showed good efficacy against pneumonic pasteurellosis in pigs, when it was compared with enrofloxacin and oxytetracycline (Chung and Yeh, 1993).

Septic shock or endotoxaemia are systemic inflammatory syndromes known to result in an activation of cascade of inflammatory mediators (Marshal et al., 1995). This condition profoundly affects circulatory system through maldistribution of blood flow.
Hypoperfusion is due to diminished central and impaired regional circulation. The effects on central circulation include depressed myocardial contractility, hypovolaemia and pronounced vasodilation. The reduced cardiac output and local vasoconstriction results in impaired regional blood flow and also disturb the end-organ function that culminates in multiple organ failure (MOF) which is the leading cause of morbidity and mortality. Recent studies have supported the concept that an early reimbursement of the circulation improves cardiac output and oxygenation that ultimately increases the survival rate (Rivers et al., 2001). Therefore, it is important to restore the intravascular volume and thereby increase cardiac output (CO) and oxygen delivery (Bone et al., 1997; Somel et al., 2005). The debate on which fluid to use for resuscitation is still ongoing, and both crystalloid and colloid solutions are advocated (Schierhout and Roberts, 1998).

2.1.1. Pathophysiology of Septic Shock

Most cases of the septic shock (approximately 70%) are caused by endotoxin-producing gram-negative bacilli, hence the term endotoxic shock. Endotoxins consists of components of the gram-negative bacterial cell wall lipopolysaccharides (LPS) that are released when the cell walls are degraded (e.g., in an inflammatory response). Lipopolysaccharide consists of two layers, an inner phospholipids bilayer intersected by transport proteins having toxic fatty acid (lipid A) core and an outer layer of a complex polysaccharide coat (including O antigens) unique to each bacterial species. The pathophysiological changes in sepsis are exerted by lipid A component of the LPS (Rietschel et al., 1993). Contrarily, exotoxins are the main dependant part of gram-positive bacteria which act as superantigens to initiate the process of the sepsis syndrome (Idanpaan-Heikkila and Tuomanen, 1997).

In the bloodstream, free LPS attaches to a circulating LPS-binding protein, this leads the endotoxins to immunocompetent cells (macrophages) which are the central in inflammation. The LPS-binding protein complex interacts with the membrane bound surface protein CD14 (Tobias et al., 1995). This ligand complex then attaches to a signal-transducing protein called mammalian Toll-like receptor protein 4 (TLR-4) (Opal and Esmon, 2003). Signals from TLR-4 can then directly activate cells of vascular wall and
leukocytes with subsequent activation of inflammatory and immune responses (Bone et al., 1997). The host response to endotoxins has evolved for protection against infection, but the pathophysiological changes propagated as the septic shock develops.

Engagement of the TLR-4 receptor on monocytes and macrophages causes profound mononuclear cell activation with the subsequent production of a cascade of cytokine mediators, which recruit neutrophils, jointly with a variety of other inflammatory molecules. Presumably this series of responses efficiently eradicate invading microbes by triggering elements of the innate immune system and producing highly toxic and unstable oxygen derivatives. The initial pro-inflammatory cytokine response includes tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6) and platelet agglutination factor (PAF). Starting with TNF they are produced in series of one another and are often referred to as a "cytokine cascade". The principal inducer of physiologic TNF-α is lipopolysaccharide (LPS) which was originally described as the primary mediator of septic shock (Vilcek and Lee, 1991; Welboum et al., 1991; Blackwell and Christman, 1996). Tumour necrosis factor-α synthesis and release has been shown able to reproduce most of the toxic effects induced by endotoxins (Beutler and Kruys, 1995), with subsequent production of the other interleukins. To balance the resulting inflammation, anti-inflammatory cytokines are produced such as interleukin-4 (IL-4) and interleukin-10 (IL-10). The different cytokines are ascribed various and specific effects such as including fever, capillary leak and vasodilation. Unfortunately, depending on the dosage and numbers of macrophages that are activated, a cascade of other inflammatory molecules and systems participate in the inflammatory response, leading to the different pathophysiological changes including fatal shock. Such molecules are nitric oxide (NO), products of arachidonic acid metabolism and toxic oxygen free radicals (Gomez-Jimenez et al., 1995).

The higher level of LPS supervene the septic shock syndrome and result in haemodynamic derangements which are characterized by systemic vasodilation with concomitant hypotension, diminished myocardial contractility resulting in reduced systemic vascular resistance, in the presence of an increased or normal cardiac output (Wilson and Brackett, 1983; Groeneveld et al., 1986). Hypovolaemia aggravates with the
progress in septic shock by fluid loss from the intravascular space due to increased capillary permeability which augments hypotension. In this later phase of shock, because of hypoperfusion resulting from the effects of widespread vasodilation, cardiac output fails and abnormal distribution of systemic and microvascular blood flow further limits effective nutritional blood flow (Court et al., 2002).

The myocardial pump failure and vascular changes result in impaired oxygen delivery to peripheral organs with ensuing organ damage. The oxygen demand of the cells increased due to impaired oxygen delivery which augments the imbalance between cellular oxygen demand and delivery resulting in anaerobic metabolism and lactate formation. This cytopathic dysoxia further impairs cellular metabolism, ultimately causing cell death. The resulting organ damage may affect several organs, leading to multiple organ dysfunctions (MOD). In sepsis, acute lung injury occurred due to pulmonary hypertension, depreciated gas exchange and lung compliance due to oedema and neutrophil sequestration (Astiz and Rackow, 1997).

2.1.2. Fluid Therapy in Sepsis

Systemic inflammatory response and alterations in the endothelial barrier result in hypovolaemia in the absence of obvious fluid loss. Thus, treating hypovolaemia with adequate fluid therapy appears to be a mainstay of managing the critically ill septic patient (Dellinger et al., 2004). It is, therefore, of great importance to improve cardiac output and microcirculatory perfusion through restoration of intravascular volume with the help of fluids administration, hence, prevent the development of multiple organ failure (Rivers et al., 2001; Durairaj and Schmidt, 2008). The debate on the use of fluid for resuscitation is still ongoing, and both crystalloids and colloids are advocated (Schierhout and Roberts, 1998; Choi et al., 1999).

2.1.3. Crystalloids versus colloids

A crystalloid is a solution of small, ionic or non-ionic having low molecular weight. The solution freely moves across the vascular membrane and distributes throughout the extracellular fluid space, thus making the effect of resuscitation transient,
as the solution shifts from the intravascular space. Crystalloid fluid therapy, therefore, requires a large volume of replacement fluid. This may be associated with the risk of accumulation of excess fluid in interstitial tissue resulting in oedema. Examples are isotonic saline solution, Ringer’s lactate and Ringer’s acetate (Choi et al., 1999; Prough and Svensen, 2006).

A colloid is a solution of high molecular weight that largely remains in the intravascular compartments, thereby contributing to the oncotic pressure, leading to the plasma volume expansion. The degree of volume expansion is mainly determined by the molecular weight of the colloid substance, making colloids more effective volume expanders compared to the same volume of the crystalloids. The examples are both natural (albumin and plasma) and artificial (dextran, starch and gelatin) forms of colloids. Reported adverse effects of colloids are alteration of coagulation, affected renal function and anaphylactic reactions (Schierhout and Roberts, 1998; Dubois and Vincent, 2006).

2.2. Calf Scour

According to Roy (1983) the neonatal period extends from birth to twenty eight days (4 weeks) of life. During this period, mortality rate ranges from 7.10 to 51.72% (Roy, 1983). Total mortality rate is as high as 84% in the first month of age (Jenny et al., 1981). In the first month of age, particularly during third week of life, mortality is as high as 84% of the total mortality (Jenny et al., 1981; Umoh, 1982). High mortality among neonatal calves is a serious concern in dairy herd and is commonly attributed to disease conditions like diarrhoea, pneumonia and pneumo-enteritis (Khan and Khan, 1992; Khan and Khan, 1996), of these diarrhoea is the leading cause for morbidity and mortality.

Calf scour (neonatal calf diarrhoea) is one of the most important causes of death (Gitau et al., 1994) and economic loss to livestock industry (Khan and Khan, 1992). There are certain factors that predispose young calves to infectious agents causing diarrhoea. These factors include:
2.2.1. Season

The mortality in buffalo neonates in relation to season of the year is higher during summer (Khan and Khan, 1995). In Punjab province of Pakistan, the summer extends from April to September. Early summer has high dry heat and less humidity, while late summer becomes more hot and humid. Increased humidity provides favourable environment to the aetiological agents to affect neonatal calves and cause diarrhoea. Pyne and Gupta (1992) also reported high mortality during summer rainy season.

2.2.2. Age

The mortality in relation to age of neonate was the highest during 3rd week. *Salmonella* was found to be the most frequent aetiological agent during 3rd week of life (Greene and Dempsy, 1986). The incidence of enteropathogenic *E. coli* was more in 1st week and decreased in 2nd and 3rd week (Radostits *et al*., 2007).

2.2.3. Birth weight

Weight of the calf is another factor which is reported to influence on the calf mortality. If body weight of the neonate calf is higher than 20 kg, reduced mortality was observed (Roy *et al*., 1997). However, Khan and Khan (1996) failed to establish relationship of mortality with body weight of neonatal buffalo calves.

2.2.4. Treatment

The aetiological agents causing neonatal calf diarrhoea include enteropathogenic *Escherichia coli, Salmonella spp., rotavirus, coronavirus* and *cryptosporidium* (Radostits *et al*., 2007). Bacterial organism found in faecal samples of diarrhoeic buffalo neonates showed the highest incidence of enteropathogenic *E. coli* (55.6%) followed by *Salmonella* spp. (13.4%) (Tooba, 2001). Pathophysiology of *E. coli* like those of other gram-negative bacteria is mediated by the release of endotoxins subsequent to death of bacterial cells (Radostits *et al*., 2007). These endotoxins result in endotoxic shock. The manifestations like dehydration, acidosis and electrolyte imbalance are due to these endotoxins and are common causes of death (Constable *et al*., 1991a; Walker *et al*., 1998; Senturk, 2003; Flores *et al*., 2006). Mostly, dehydrations in calves with neonatal diarrhoea are hyponatremic (Constable, 1999; Flores *et al*., 2006), with decreased
extracellular and increased intracellular fluid. Severely dehydrated calves that are unable to suckle need intravenous fluids for resuscitation. To fulfill the requirement of lost body electrolytes is the basic tenet for the successful treatment of diarrhoeic calves that primarily depends on the administration of fluids. Administration of a correct fluid in right amount and at right time may make a difference between life and death. For this purpose, various treatment regimens have been tried on neonatal calves suffering from diarrhoea.

A solution composed of 8.4% bicarbonate solution was used successfully for treating acidosis in calves with diarrhoea (Glawisching et al., 1990). Neonatal calf diarrhoea treated with danofoloxacin was recovered in the form of improved clinical condition, increased weight gain and less mortality compared to placebo and oxytetracyclin (Holck et al., 1994). Lofstedt et al., (1996) compared the efficiency of sulbactam:ampicillin to that of ampicillin trihydrate and 0.9% saline for the treatment of diarrhoea in calves induced by oral inoculation with E. coli strain B44 (09:K30:K99:H). Treatment was initiated when severe diarrhoea was noted and continued for at least three days; or for 24 hours after clinical signs resolved; or for a maximum duration of seven days. Twenty four hours after treatment, mean rectal temperature of sulbactam:ampicillin calves was significantly higher than that of saline treated calves; eye position and skin elasticity score of former was significantly lower than those of latter and ampicillin treated calves.

Groutides and Michell (1990) compared five commercially available parenteral solutions for their effectiveness in correcting the disturbance associated with diarrhoea induced by E. coli. Each solution (saline, Hartmann’s, Darrow’s, Plasmlyte and glucose) was tested on 8 Jersy calves less than a week old and weighing approximately 25 kg. Each calf received 8.6 litres over 3 days, at the rate of 20 ml/kg/h. Solution such as saline or plasmalyte which had higher concentrations of sodium were more effective at correcting dehydration and electrolyte disturbances than those with less sodium (Darrow’s, Hartmann’s). It was further stated that only those with bicarbonate precursors (lactate, acetate, gluconate) were effective in correcting metabolic acidosis.
Grove and White (1993) evaluated the efficacy of intravenous fluid therapy in the treatment of acidosis in 32 calves. All the calves were infused with 5-10 litres of electrolyte solution containing 144 mmol/litre sodium, 4 mmol/litre potassium, 113 mmol/litre chloride and 35 mmol/litre bicarbonate. Nearly all the calves were recumbent but less than half were dehydrated on admission. The signs of dehydration were well correlated with haematocrit. All 32 calves were recovered from acidosis and dehydration with the help of infusion of intravenous fluid therapy. The calves treated with crystalloid and colloid gave best results (Hartmann and Reder, 1995). Rossow et al., (1994) recommended a continuous intravenous infusion of 40% glucose and 0.9% saline solution and recovered the calves more efficiently with normal saline as compared to glucose.

Hypertonic saline solution (7.2% NaCl) was used in the resuscitation of diarrhoeic calves by Constable and his colleagues (1996) for the first time. He determined its resuscitative effectiveness in combination with dextran in 16 induced diarrhoeic and hypovolaemic calves. The diarrhoea was induced by the oral administration of milk replacer @ 33 mL/Kg BW and isotonic sucrose solution @ 2 g of sucrose in 19.5 mL of water/Kg every eight hours, along with furosemide @ 2 mg/Kg, IM, every four to eight hours. He concluded that combined administration of hypertonic saline and dextran solution along with oral electrolyte solution caused immediate and sustained effects in the resuscitation of diarrhoeic calves. In another study, Walker et al., (1998) compared the effects of hypertonic saline dextran solution (HSD) with lactated Ringer’s solution for resuscitating severely dehydrated calves with diarrhoea. Calves were induced diarrhoea with the same protocol as adopted in previous studies (Constable et al., 1996). They concluded that the combination of HSD and oral electrolyte solution was a rapid and effective method for the resuscitation of severely dehydrated calves and showed similar effectiveness as conventional treatment in which lactated Ringer’s and oral electrolyte solutions were used.

Senturk (2003) studied the effects of small-volume IV 7.2% NaCl in combination with 6% dextran-70 solution along with oral fluid treatment (50 ml/kg, PO) and compared it with isotonic saline solution along with oral fluid treatment (22 ml/kg, PO) in thirty dehydrated diarrhoeic calves of age 2–45 days. In comparison with isotonic
saline, hypertonic saline was observed to have beneficial effects in the resuscitation of dehydrated diarrhoeic calves. Administration of small-volume of hypertonic saline and dextran-70 solution in combination with oral electrolyte solutions significantly (P < 0.001) shortened capillary refill time and improved peripheral pulse quality. Moreover, the degree of dehydration was also significantly improved.

Flores et al., (2006) compared the effects of IV administered hypertonic (7.2% NaCl) and isotonic (0.9% NaCl) saline solutions in combination with oral rehydrating solution in sixteen osmotic diarrhoea-induced male Holstein calves from 1 to 9 days old. Hypertonic saline solution restored the plasma volume, serum sodium and chloride concentrations in a single dose when compared to isotonic saline. Hypertonic saline also reestablished serum potassium concentration and glomerular filtration rhythm. They concluded that bolus administration of hypertonic saline solution along with oral rehydrating solution increased plasma volume, sodium and chloride concentrations, serum osmolality, glomerular filtration rhythm immediately. It also restored extracellular fluid volume constituting a practical and economical alternative to the use of large volumes of isotonic saline solution.

Bleul et al., (2007) determined efficacy of 5% sodium bicarbonate (NaHCO$_3$) solution in 20 newborn calves with acidosis (blood pH < 7.2) and compared with isotonic saline solution. The dose of hypertonic solution infused was based on the severity of the acidemia. Administration of hypertonic NaHCO$_3$ solution to neonatal calves with metabolic acidosis did not have any adverse effects on plasma concentrations of several commonly measured electrolytes or enzyme activities. The volume of hypertonic NaHCO$_3$ used was smaller compared with that of isotonic saline solution and could be used effectively in field settings.

Koch and Kaske (2008) compared the efficacy of IV administered hypertonic saline (HSS; 5.85% NaCl @ 5 mL/Kg BW) and hypertonic bicarbonate (HBS; 8.4% @ 10 mL/Kg BW) solutions followed by oral administration of 3L isotonic electrolyte solution in the treatment of diarrheic calves. They hypothesized that HBS is more advantageous than HSS in the resuscitation of calves with metabolic acidosis. For this
purpose, 28 dehydrated calves with neonatal diarrhea were used. They concluded that diarrhoeic calves with metabolic acidosis could be successfully resuscitated with hypertonic saline, while HBS was appropriated in calves with more severe metabolic acidosis.

2.3. Hypertonic Saline Solution

Hypertonic saline solution (HSS) have been interestingly explored as possible resuscitative fluid for hypovolaemia since 1919, when Penfield published a report revealing that a small volume of 1.8% NaCl solution induced a transient recovery of the hypotension caused by blood loss in dogs (Penfield, 1919). Later, a 5% NaCl solution was clinically applied in the treatment of Buerger’s disease (Silbert, 1926). No other noticeable data on use of HSS emerged in literature till the sixties, when Brooks et al. (1963) reported that HSS prevented organ damage and provided rapid recovery in dogs subjected to haemorrhagic shock. Baue et al. published two reports in year of 1967 on beneficial effects of 5% NaCl solution on oxygen consumption and metabolic acidosis. In 1969, Messmer et al. demonstrated equally transient effects of 7.5% NaCl in severely hemorrhaged dogs. In the same year, Gazitua et al. described that hyperosmotic NaCl solution induced vasodilation when selectively infused in the renal, coronary, and limb circulations.

However, concept for the use of hypertonic saline solutions for the treatment of shock got strengthen when Velasco and colleagues reported that dogs subjected to severe haemorrhagic shock were resuscitated successfully with an increased arterial blood pressure and improved cardiac output following IV bolus injections of 7.5% NaCl @ 4 ml/kg BW, a volume equivalent to only 10% of the volume of shed blood. Long term survival was observed in all 10 HSS treated dogs; in contrast, control animals, treated with an equal volume of isotonic saline, responded with neither haemodynamic improvement, nor survival. After successful introduction in the resuscitation of circulatory collapse in animal model, hypertonic saline (7.5% NaCl) solution was successfully pioneered to induce haemodynamic benefits in humans at an emergency intensive care unit in refractory shock by Fellippe et al. (1980). After publishing of these
reports, many studies were conducted all over the world, including experimental and clinical trials of hypertonic NaCl in the treatment of hypovolaemic and endotoxic shock. Several research groups confirmed the findings of Velasco et al. that a small volume of 7.5% NaCl rapidly restored arterial pressure, cardiac output and blood flows to vital organs (Nakayama et al., 1984; Smith et al., 1985; Kramer et al., 1986; Rocha e Silva et al., 1986; Poli de Figueiredo et al., 1995). After the systemic studies on the successful resuscitative response with a low volume of HSS it was named as “small-volume resuscitation”. This term for hypertonic saline solution was coined by Nakayama and his colleagues in 1984.

In the mid eighties, Kramer and his colleagues (1986) added new conceptual and practical possibilities by combining the hyperosmotic effects of NaCl to the hyperoncotic effects of dextran and used 7.5% saline mixed with 6% dextran-70 (HSD). It was hypothesized that association of dextran to the HSS enhances the initial plasma expansion. However, the most important effect of HSD is the maintenance of the intravascular expansion for longer period, thus, helps in prolonging the haemodynamic and metabolic benefits (Kramer et al., 1986; Velasco et al., 1989; Walsh and Kramer, 1991). Holcroft et al. (1987) were the first authors who successfully applied the concept of small-volume resuscitation with dextran to preclinical trauma care. Their data showed a higher arterial pressure and survival rate after an initial resuscitation using hypertonic saline with colloid compared with isotonic crystalloid resuscitation only. The present review and study, however, concentrate on the effects of pure HSS alone.

2.3.1. Physical properties of hypertonic saline solution

Hypertonic saline solution is intended for primary resuscitation by instituting a high osmotic gradient across cellular membranes which cause shifting of endogenous water into the intravascular compartment that ultimately resulted in expansion of plasma volume. The recommended dose of 7.5% NaCl (4 ml/kg) adds a 5.12 mmol load of sodium/kg body weight (Constable et al., 1991a,b; Rocha e Silva and Poli de Figueiredo, 2005). The beneficial haemodynamic effects of HSS could be obtained with rapid plasma volume expansion, a vagally mediated reflex dependant upon stimulation of pulmonary osmoreceptors and increased cardiac contractility (Velasco et al., 1980; Lopes et al.,
1981; Younes et al., 1985). In addition, HSS entails no risks of allergic reactions and transmission of infectious agents with its administration when compared with artificial plasma expanders and human plasma (Vassar et al., 1990).

2.3.2. Physiological properties of hypertonic saline solution

Administration of 4 ml/kg of 7-7.5% sodium chloride solution improve partial recovery of mean arterial pressure and cardiac output that has been attributed to plasma volume expansion, vasodilatation in several vascular beds (Gazitua et al., 1969) and to a direct cardiac inotropic effect (Rocha e Silva et al., 1987; Kreimeier et al., 1990).

Effects on microcirculation

Generally, the infusion of hypertonic solution induces changes on the peripheral vasculature and microcirculation that enhance flow. These changes are observed in result of reduced peripheral vascular resistance primarily caused by arteriolar vasodilation. Increased osmolarity due to hyperosmotic fluid infusion effects directly in the relaxation of vascular smooth muscle (Gazitua et al., 1971). Haemodilution causes reduction in blood viscosity which also contributes to the decrease in calculated peripheral vascular resistance (Mazzoni et al., 1988).

The hypovolaemic or endotoxic shock induced hypoxia and activation of polymorphonuclear cells in the endothelium which produce oedema of endothelial cells and the erythrocytes which are of critical importance in terms of capillary flow. This leads to capillary lumen narrowing, thereby causing obstruction in blood flow, so, increasing viscosity and hydraulic resistance and reduction in oxygen transport. Under these circumstances, cellular oedema compromises the microcirculatory blood flow. Small volume resuscitation instantly corrects this geometric anomaly primarily through mobilizing the intracellular fluid from microvascular endothelial cells and erythrocytes. As oedema is greater in capillaries, so, more pronounced effects are seemed in capillaries. It produces a reduction in hydraulic resistance and an improvement in tissue perfusion (Mazzoni et al., 1988; Mazzoni et al., 1990).

The classical papers of Mazzoni et al. (1988 and 1990) described that oedematous erythrocytes and endothelium lose 20% of their volumes directly to the intravascular
compartment mainly on account of their immediate physical contact with the intravascular hypertonicity after infusion of 7.5% NaCl solution. So, the main sources for the plasma volume expansion are the erythrocytes and the endothelium. Apart from the volume expansion, this fluid shift has important haemodynamic consequences, at the microcirculatory level.

**Plasma volume expansion**

Many researchers have reported that infusion of a small volume of hypertonic solution (7-7.5% NaCl) induces expansion in plasma volume (Nakayama et al., 1984; Smith et al., 1985; Rocha e Silva et al., 1987; Schmall et al., 1990; Constable et al., 1991a). Many studies have reported that a redistribution of fluids from the perivascular to the intravascular space occur which consequently expand plasma volume (Velasco et al., 1980; Younas et al., 1985; Velasco et al., 1989). The volume expansion is two to four times the infused volume (Velasco et al., 1989; Kramer, 2003). Osmotically drawing volume expansion of intracellular and interstitial water into the vascular space is approximately 3 mL for every 1 mL of hypertonic saline infused (Constable, 1999). As measured by the dye dilution technique in unanaesthetized sheep, the plasma volume expansion after a blood loss of 1.6 litres and infusion of 160 ml of hypertonic saline (7% NaCl) was 360 ml (Nakayama et al., 1984). Some water must, therefore, move from within cells into the extracellular space as a result of the osmotic action of the hyperosmotic solution. This explains why it takes a smaller volume of hypertonic than isotonic saline to produce similar effects (Kien et al., 1991).

Convincing study on plasma volume expansion after infusion of hypertonic saline in normovolaemic animals were conducted by Dubick et al. (1995), who made serial measurements of plasma volume using tracer dilution techniques. They demonstrated that hypertonic saline infusion showed maximum volume expansion immediately at the end of infusion. A microvascular transendothelial driving forces analysis supports the concept of immediate intravascular expansion after hypertonic saline infusion. Each milliosmole difference generates an osmotic pressure of 19.3 mm Hg at 37°C across an ideal semipermeable membrane. However, the microvascular wall is not an ideal membrane and has an osmotic reflection coefficient of 0.1 to 0.3 which results in only 10% to 30%
of the total osmotic pressure being exerted across endothelial cells, while cell membrane coefficient is 1 (Wolf and Watson, 1989). Thus, for instance, infusion of 4 ml/kg of 2400 mOsm hypertonic saline transiently increases serum osmolality by 40 to 50 mOsm across the endothelial membrane, but 500 mm Hg osmotic pressure across the cellular membrane. So, water moves rapidly enough to alleviate the force of osmotic pressure across the cell membrane to equilibrate the osmotic forces between intracellular and extracellular fluids. Thus, the intravascular expansion induced by hypertonic resuscitation occurs from the intracellular compartment and not from the interstitial compartment. This mobilized cellular water expands the interstitial space and dilutes the interstitial protein which increases the plasma-to-interstitial oncotic gradient (Wolf and Watson, 1989; Tollofsrud et al., 1998). Thus, provides the source fluid for the vascular volume expansion, increases interstitial hydrostatic pressure. Hypertonic saline infusion also increases plasma protein due to interstitial expansion which resulted in increased lymph and protein flow (Pascual et al., 1992). This fluid shift from the intracellular to the extracellular compartment and increase in plasma protein may be regarded as beneficial in hypovolaemic and septic shock (Rocha e Silva and Poli de Figueiredo, 2005).

**Cardiovascular effects**

Cardiac function could be estimated by measuring the cardiac output, cardiac index, heart rate, stroke volume, preload and cardiac contractility. Hypertonic saline solution (7-7.5% NaCl) is capable to expand plasma volume significantly which may certainly contribute to increased cardiac output. Walsh and Kramer (1991) observed increased cardiac output correlated significantly with the expansion of plasma volume.

Some studies also showed that infusion of hypertonic saline stimulate pulmonary osmoreceptors and it initiate cardiovascular reflex which plays a major role in improving the cardiac output (Lopes et al., 1981; Younes et al., 1985; Rocha-e-Silva et al., 1987). This reflex induces venoconstriction (Lopes et al., 1986) and selective muscular and cutaneous precapillary constriction (Rocha-e-Silva et al., 1986), which would redistribute the blood flow, allowing an increase in cardiac output. In addition to the expansion of the plasma volume and the pulmonary reflex, increased stroke volume, cardiac contractility,
and heart rate might also contribute to the increased cardiac output (Constable et al., 1991a).

Bertone and colleagues (1990) found that hypertonic saline had ability to promote a more profound and longer-lasting cardiovascular normalization when compared with isotonic saline injected at the same rate (5 ml/kg). Similarly, Mullins and Hudgens (1987) suggested that the combination of the hyperosmotic mechanisms induced by hypertonic solutions, with water transfer from the extravascular to the vascular compartment, is equivalent to the effect produced when a large volume of isotonic solution is administered.

**Immunomodulatory effects**

Sepsis often initiates a systemic inflammatory response accompanied by multiple organ failure. Lungs are the foremost and common targeted organ in the models of haemorrhagic and septic shock because of neutrophils sequestration in the lungs (Botha et al., 1995; Angle et al., 1998). Hypertonic saline has been shown to reduce lung injury after haemorrhagic shock (Angle et al., 1998; Rizoli et al., 1998; Rizoli et al., 2006). These studies showed that HSS reduced the neutrophil accumulation in the lungs and there were less neutrophils recovered on bronchoalveolar lavage and histopathology showed lower degree of lungs injury. Inflammatory condition promotes the neutrophils to adhere the endothelial via CD11b integrin. In this respect, Rizoli and co-workers (1998) showed that HSS inhibited the activation of CD11b and prevented lipopolysaccharide-stimulated expression. This suggests that HSS reduces lung injury by preventing neutrophil adhesion to endothelium.

**2.3.3. Adverse effects of hypertonic saline solution**

Different concentrations of sodium chloride (up to 30 % NaCl) for hypertonic solutions have been studied in the controlled studies for the resuscitation from haemorrhagic shock. Bolus administration of 10% NaCl solution into the peripheral vein resulted in significant haemolysis (Rocha e Silva et al., 1990), while 30% NaCl caused hypernatremia along with haemolysis, whereas, 7-7.5% NaCl are regarded as safe (Rocha e Silva and Poli de Figueiredo, 2005).
3.1. Evaluation of Hypertonic Saline Solution in the Treatment of Haemorrhagic Septicaemia

3.1.1. Study Animals

A total of 50 buffaloes of either sex suffering from haemorrhagic septicaemia were selected from the field. The disease was diagnosed on the basis of clinical signs i.e. acute, elevated body temperature (105-107 °F), dullness, anorexia, difficult breathing, nasal discharge, hypersalivation, reluctance to move, swelling on throat, brisket and upper dewlap region (Carter and De Alwis, 1989; Raza et al., 2000; Radostits et al., 2007). The age spectrum of animals ranged from 06 months to 2 years.

3.1.2. Instrumentation

Intravenous catheters were placed aseptically in right and left jugular furrow after clipping the hair. For infusion of solutions and collection of blood samples, catheter of 14-gauges 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). The area over auricular artery was shaved and aseptically prepared for cannulation of auricular 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 (PaO₂). 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 auricular artery’s catheters were flushed with heparinized saline solution containing 40 IU/mL before measuring the CVP and MAP, respectively.
Uniformity of animals with respect to disease severity in both the groups

The severity of the symptoms of disease was monitored in each case and recorded carefully before allotting the treatment. Clinical measurements such as dullness, body temperature, swelling, salivation, respiratory rate, nasal discharge and feed consumption were clinically evaluated in weighted scores as mild (2), moderate (4) and severe (6).

Table 1. Clinical scoring system and weighed score for calculation of the severity index of haemorrhagic septicaemia in buffaloes

<table>
<thead>
<tr>
<th>Clinical Measurements</th>
<th>Severity score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>1. Dullness</td>
<td>2</td>
</tr>
<tr>
<td>2. Temperature</td>
<td>2 (39.4 - 40 °C)</td>
</tr>
<tr>
<td>3. Swelling</td>
<td>2</td>
</tr>
<tr>
<td>4. Salivation</td>
<td>2</td>
</tr>
<tr>
<td>5. Respiration rate</td>
<td>2 (14-18/min)</td>
</tr>
<tr>
<td>6. Nasal discharge</td>
<td>2</td>
</tr>
<tr>
<td>7. Feed consumption</td>
<td>2</td>
</tr>
</tbody>
</table>

Weighed Score 14 28 42

3.1.3. Experimental Design

On meeting the aforementioned criteria for entering the treatment phase of the study, buffaloes suffering from disease were randomly divided through random number table (Chow and Lin, 2004) into two equal groups i.e. group A and group B.

Group A (n=25) served as control and was treated with conventional treatment already in vogue i.e. ceftiofur HCl (Excenel RTU®, Pfizer Animal Health Division,
Pakistan) @ 6 mg/kg body weight (BW), intramuscular (IM) along with intravenous administration of flunixin meglumine (Loxin®, Selmore Pharmaceuticals, Pakistan) @ 2 mg/kg BW.

**Group B** (n=25) was experimental group and treated with bolus intravenous infusion of hypertonic saline solution (7.5% NaCl; 2400 mmol NaCl/L) @ 4 mL/kg BW followed by normal saline solution @ 10 mL/kg BW in combination with ceftiofur HCl IM @ 6 mg/kg BW and flunixin meglumine IV @ 2 mg/kg BW.

In both the groups, the treatment started after application of cold water on the head of each buffalo for 15 minutes to lower the body temperature. Hypertonic saline solution was administered once in the buffaloes of group B during a 8-minute period via the jugular catheter. The HSS provided 4.9 mmol of sodium/kg to each buffalo. Ceftiofur HCl and flunixin meglumine were repeated at every 12 and 8 hours, respectively.

**Measurement and Analysis of Samples**

The evaluation criteria and recording intervals are described in details in section 3.4.

**3.2. Evaluation of Hypertonic Saline Solution in the Treatment of Induced Calf Scour**

**3.2.1. Experimental Animals**

The experiment was conducted in accordance with a protocol approved by the Animal Ethics Committee, University of Agriculture, Faisalabad, Pakistan. The study was conducted on 24 university-owned, colostrum-fed healthy male buffalo calves of 3-5 days old and with a mean weight of 40 ± 4.5 kg. Animals underwent a 7 days acclimatized period. These calves were maintained at Livestock Research Farms, University of Agriculture, Faisalabad, Pakistan. Animals were sheltered in individual movable iron pens 30 cm over the ground and with wooden-strip floor. The diet consisted of powdered milk dissolved in water administered PO through bottles with nipple, bid @ 10 percent of body weight per calf per day.
3.2.2. Instrumentation

The day prior to induction of scour, 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 furrow were clipped and the skin was surgically scrubbed for aseptic placement of IV catheters. For infusion of solutions and collection of blood samples, catheter of 18-gauges 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 aseptically prepared for cannulation of femoral artery with the help of 20-gauge catheter for the measurement of mean arterial pressure (MAP) and collection of blood samples for determination of partial pressure of arterial oxygen (PaO\textsubscript{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/ml before measuring the CVP and MAP, respectively. Xylazine sedation was reversed by administering tolazoline HCl (1 mg/kg) after catheterization. The calves were placed in moveable stalls for at least 12 hours after instrumentation. Baseline values were then recorded by obtaining blood samples.

3.2.3. Induction of Diarrhoea

An enteropathogenic Escherichia coli isolated from a field case of neonatal buffalo calf diarrhoea was procured from National Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan. Infection was introduced through oral administration of 2 ml broth culture having E. coli count of $10^{10}$ CFU (Colony Forming Units) of E. coli dissolved in normal saline to each calf of both the groups (Brooks et al., 1996). Twenty four hours after the onset of diarrhoea, calves of both the groups were considered for the treatment phase.

3.2.4. Experimental Design

At the start of treatment phase, calves were randomly allocated 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 the control group (Group A) were infused intravenously with normal saline (0.9% NaCl) solution @ 90 mL/kg BW along with ceftiofur HCl @ 6 mg/kg BW, IM and flunixin meglumine @ 2 mg/kg BW, IV. Calves in group B were dosed intravenously with hypertonic saline (7.5% NaCl) solution @ 4 mL/kg BW during a 4-minute period via jugular vein followed by normal saline solution @ 10 mL/kg BW, in combination with ceftiofur HCl @ 6 mg/kg BW, IM and flunixin meglumine @ 2 mg/kg BW, IV.

In both the groups, the fluids were infused once at the first day treatment, while ceftiofur HCl and flunixin meglumine were repeated after 24 and 12 hours, respectively. Hypertonic saline solution provided 4.9 mmol of Na⁺/kg, while normal saline solution provided 14 mmol of Na⁺/kg to each calf of their group.

The physical condition of each calf was assessed at least every 6 hours during the study and calves that were unable to stand without assistance were not considered. At 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

The evaluation criteria and recording intervals are described in details in section 3.4.

3.3. Evaluation of Hypertonic Saline Solution in the Clinical Conditions of Livestock Characterized by Endotoxaemia and/or Hypovolaemia

This part of the study included clinical trials of hypertonic saline solution in different conditions of the food animals characterized by endotoxaemia and/or hypovolaemia. These clinical conditions included:

A). Spontaneous calf scour

B). Diarrhoeic dehydrated goats and

C). Diarrhoeic buffaloes
3.3.1. Study Animals

A. Spontaneous calf scour: This part of clinical study was carried out on 24 client-owned buffalo calves presented to the Veterinary Medical Teaching Hospital (VMTH), Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Calves were selected on the basis of following criteria: age < 25 days, 24 hours scouring before presentation for treatment (diarrhea was defined as soupy or watery consistency of the faeces), moderate to severe dehydration (skin tenting time > 3 but < 10 seconds, eyes slightly to markedly recessed into orbit), mild or no suckling reflex and no clinical symptoms of secondary organ diseases.

B. Diarrhoeic dehydrated goats: A total of 24 adult, female goats suffering from Peste des Petits Ruminants (PPR) were admitted at VMTH of the department. The goats considered for the study had severe dehydration due to diarrhoea that is a clinical symptom of PPR along with other characteristic signs such as high rise in body temperature (above 40°C), coughing, sneezing and development of necrotic lesions in mouth extended over the entire mucosa (Radostits et al., 2007).

C. Diarrhoeic buffaloes: Adult buffaloes (n=24) suffering from diarrhoea were included in the study. Diarrhoea was defined as the voiding of fluid faeces that splashed when hitting the ground, with failure to form a pasty, round buffalo pat.

3.3.2. Instrumentation

Intravenous catheters were placed aseptically in right and left jugular furrow after clipping the hair. For infusion of solutions and collection of blood samples, catheter of 14-gauges was placed in right jugular vein. Left jugular vein was catheterized with 7-F thermodilution Swan-Ganz catheter for recording central venous pressure (CVP) in calves and goats, however, this parameter was not studied in diarrhoeic buffaloes. In calves and goats, the area over femoral artery was shaved and aseptically prepared 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$_2$). Mean arterial pressure and PaO$_2$ were again not the part of diarrhoeic buffaloes study. 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/mL before measuring the CVP and MAP, respectively.

### 3.3.3. Experimental Design

Diarrhoeic dehydrated calves, goats, and buffaloes were randomly divided into two equal groups (n=12 animals/group). Animals of **group A** were infused with normal saline solution @ 90 mL/kg BW, IV, and treated with ceftiofur HCl @ 6 mg/kg BW, IM and flunixin meglumine @ 2 mg/kg BW, IV. While animals of **group B** were dosed intravenously with HSS @ 4 mL/kg BW followed by normal saline solution @ 10 mL/kg BW in combination with ceftiofur HCl @ 6 mg/kg BW, IM and flunixin meglumine @ 2 mg/kg BW, IV.

### 3.4. Measurement and Analysis of Samples

Clinical parameters (rectal temperature, respiratory rate and heart rate), haematological profiles (haemoglobin concentration, haematocrit), serum electrolytes (sodium, chloride and potassium ions concentration) and blood gas analysis [partial pressure of arterial oxygen (PaO$_2$), venous carbon dioxide (PvCO$_2$), venous blood pH and bicarbonates] were assessed in all the conditions studied i.e. haemorrhagic septicaemia, induced and spontaneous cases of calf scour (neonatal calf diarrhoea), diarrhoeic dehydrated goats and diarrhoeic buffaloes. Haemodynamic parameters (mean arterial pressure, central venous pressure) were measured in all the conditions mentioned above except diarrhoeic buffaloes. Serum biochemical profiles (total protein, albumin and globulin) were studied in haemorrhagic septicaemia and induced calf scour only.

For haematological analysis, 3 mL of blood was collected into evacuated tubes that contained calcium-EDTA and 5 mL of blood was collected into evacuated tubes without anticoagulant to harvest serum followed by centrifugation and stored at -20°C until assayed. The serum was then analyzed for sodium, chloride and potassium ions.
concentration through flame photometer and total protein, albumin and globulin through spectrophotometer.

All the mentioned parameters were recorded at:

- **Baseline**: Before initiation of the treatment (during respective disease)
- **t=1 h**: One hour after allotted treatment
- **t=3 h**: Three hours after allotted treatment
- **t=6 h**: Six hours after allotted treatment
- **t=12 h**: Twelve hours after allotted treatment
- **t=24 h**: Twenty four hours after allotted treatment, and
- **t=36 h**: Thirty six hours after treatment.

However, for induced calf scour, the additional recording time point was before induction of diarrhoea which acted as baseline; other recording time points being the same as for other conditions studied.

### 3.4.1. Clinical Parameters

Survival rate was recorded on the basis of number of revived animals. Survival as used in the context of present study refers to an HS case treated with either of the two protocols and lives at hour 36 post-initiation of treatment. Body temperature was measured through rectum. Heart rate was measured by means of thoracic auscultation. Respiratory rate was measured by counting thoracic excursions, this measurement was repeated when the animal was sniffing or vocalizing.

### 3.4.2. Haematological Parameters

Blood samples collected with EDTA were analysed by methods described by Benjamin (1978) for the proceeding parameters:

**Haematocrit**

Haematocrit (Hct) was determined by the microhaematocrit method as described by Benjamin (1978). Blood mixed with EDTA was taken up to two third in plain 75 x 1 mm haematocrit capillary tubes, which were sealed at one end with polyvinyl powder at
blood free ends and centrifuged in a microhaematocrit centrifuge machine, at 1000 rpm for five minutes. Afterward each tube was placed vertically upon the haematocrit reader chart and value was recorded in percentage.

**Haemoglobin concentration**

Haemoglobin concentration was determined by cyanmethaemoglobin method. A 20 µL blood of each animal was mixed in 5 mL Drabkin’s solution. Mixing of blood into the Drabkin solution developed colour. The absorbance was measured on a spectrophotometer at 540 nm (Benjamin, 1978). Haemoglobin concentration was calculated by the following formula:

\[
\text{Conc. of haemoglobin} = \frac{\text{Absorbance of sample} \times \text{Conc. of standard}}{\text{Absorbance of standard}}
\]

**3.4.3. Haemodynamic Parameters**

Mean arterial pressure and central venous pressure were measured for the assessment of haemodynamic stability of the animals.

**Mean arterial pressure (MAP)**

Saline manometer was used to measure mean arterial pressure (Lumb and Jones, 1984). For this purpose, auricular and femoral arteries were selected for the measurement of MAP in buffaloes suffering from HS and small ruminants (calves and goats), respectively. These arteries were exposed surgically through local infiltration of Lignocaine-2% at their respective sites. Then a small nick with scalpel blade was made on the skin just over the proposed site of puncture and respective artery was located by feeling the pulse. After clearing the artery, a plastic over-the-needle type of catheter was introduced into the lumen of the artery. Next, after complete withdrawal of the needle part, a sterile stopcock was attached to the hub of the catheter and the system flushed with heparin-saline solution (40 units/mL) before recording the value of MAP through saline manometer. The catheter was fixed in position by suturing it to the skin.
Central venous pressure (CVP)

Central venous pressure was determined by connecting a graduated pipette to the proximal port of the Swan-Ganz jugular catheter, with the scapulohumeral joint serving as the 0 pressure reference point. Measurements were taken with the animal standing or in sternal recumbency, with its head in a typical alert, non-feeding, forward-facing position. Change in height of the column (cm of H₂O) was determined (Walker et al., 1998). The catheter was always flushed with heparin-saline solution (40 units/mL) before recording the value of CVP.

3.4.4. Blood Gas Analysis

Blood samples for gas analysis were obtained in 1-mL heparin-coated evacuated tubes. For determination of partial pressure of arterial oxygen (PaO₂), blood was collected from respective artery i.e. auricular or femoral, while from jugular vein to determine partial pressure of venous carbon dioxide (PvCO₂), pH and bicarbonates. These blood samples were also taken at the same intervals as other parameters. The tubes were placed on ice and processed within an hour of collection. Blood gas analysis was done with the help of automatic gas analyzer (Medica Corporation, Bedford, UK) at 37 °C. Values were automatically corrected to rectal temperature.

3.4.5. Serum Electrolytes

Serum samples stored at -20 °C were subjected to serum electrolytes determinations as under:

a. Sodium ions concentration
b. Chloride ions concentration
c. Potassium ions concentration

Wet Digestion of Samples

Wet digestion of samples was done according to the protocol described by Richard (1968). Serum sample (0.5 mL) was taken into a digestion flask and 5 mL nitric acid was added in it. The contents of the flask were placed on a hot plate for 10-15
minutes till all fumes evaporated from the flask. This left approximately 1-2 mL material in the flask. After cooling, 2.5 mL perchloric acid (HClO₄) was added. The contents of the flask were heated vigorously till volume reduced to 1-2 mL. The contents were then filtered and diluted to make a total volume of 25 mL by adding distilled water and stored in plastic bottles. These digested and diluted samples were used for the estimation of serum sodium, chloride and potassium concentrations through flame photometer.

3.4.6. Serum Biochemical Profiles

Total serum protein, serum albumin and globulin were measured among serum biochemical profiles.

Total serum protein

Total protein was determined by using Biuret reagent as described by Oser (1976). In this method, copper sulfate in the reagent reacts with peptide bonds present in protein and imparts dark blue color, which in turn is measured by spectrophotometer.

Procedure

Procedure has been described in details in table below:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>10 µL</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Standard serum</td>
<td>-----</td>
<td>10 µL</td>
<td>-----</td>
</tr>
<tr>
<td>Serum sample</td>
<td>-----</td>
<td>-----</td>
<td>10 µL</td>
</tr>
<tr>
<td>Biuret (working)</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
</tr>
</tbody>
</table>

- Took 2 mL of working Biuret reagent into three test tubes as B, S and T.
- Added 10 µL of distilled water, standard and test sample into each test tube, respectively.
- Mixed contents thoroughly and incubated for 15 minutes at 37°C.
- Placed the blank solution into blank column within spectrophotometer and adjusted reading at zero.
Fed concentration of standard into spectrophotometer and measured absorbance of standard at wavelength 540 nm (530-560nm) against blank solution. A working curve appeared on LCD screen.

Measured the concentration of test samples (g/dL) under this working curve at same wavelength against blank. Readings was completed within one hour.

**Serum albumin**

Albumin was determined by Bromocresol green dye binding method as described by Varley *et al.* (1980). Bromocresol green dye (BCG) binds with albumin and imparts green colour. The intensity of colour increases with the increase in serum albumin contents. This colour development is measured by spectrophotometer.

**Reagents:**

**Solution 1**

**Succinate buffer (pH 4.0, 1 mol/l)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic acid</td>
<td>17.715 g/1500 mL</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>2.4 g/600 mL</td>
</tr>
</tbody>
</table>

Mix both the solutions.

**Solution 2**

**Bromocresol green solution (0.58 mmol/L)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>0.4 g/100 mL D. water</td>
</tr>
<tr>
<td>Bromocresol green dye</td>
<td>419 mg</td>
</tr>
<tr>
<td>NaOH (from above solution)</td>
<td>10 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>To make 1 liter</td>
</tr>
</tbody>
</table>

**Solution 3**

**Brij 35 solution**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brij 35</td>
<td>30 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 mL</td>
</tr>
</tbody>
</table>
**Working Reagent**

Bromocresol green solution (2)  =  250 mL

Succinate buffer (1)    =  750 mL

Brij 3 solution (3)    =  7 mL

*Note:* Adjust pH to 4.2 ± 0.05

**Procedure**

Procedure has been described in details in table below:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>10 µL</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Standard serum</td>
<td>-----</td>
<td>10 µL</td>
<td>-----</td>
</tr>
<tr>
<td>Serum sample</td>
<td>-----</td>
<td>-----</td>
<td>10 µL</td>
</tr>
<tr>
<td>BCG (working)</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
</tr>
</tbody>
</table>

- Took 2 mL of working BCG reagent into three test tubes labelled as B, S and T.
- Added 10 µL of distilled water, standard and test sample into each test tube, respectively.
- Mixed contents thoroughly and incubated for 15 minutes at 37°C.
- Placed the blank solution into blank column within spectrophotometer and adjusted reading at zero.
- Fed concentration of standard into spectrophotometer and measured absorbance of standard at wavelength 600 nm against blank solution. A working curve appeared on LCD screen.
  
  The concentration of test samples (g/dL) was measured under this working curve at same wavelength against blank. Readings were completed within one hour.

**Serum globulin**

Serum globulin concentration was calculated by subtracting albumin from total serum protein (Benjamin, 1978).
3.5. 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, 2001).
Plate 1. A buffalo suffering with clinical pneumonic HS.

Plate 2. A buffalo heifer with drooling of saliva during the clinical pneumonic HS.
Plate 3. A buffalo heifer with a typical throat swelling during clinical HS. Note the encroachment of swelling on pharyngeal area.

Plate 4. Cold water irrigation of head of a buffalo heifer to lower the body temperature before initiation of treatment.
CHAPTER 4

RESULTS

4.1. Evaluation of Hypertonic Saline Solution in the Treatment of Haemorrhagic Septicaemia

The study was conducted on fifty buffaloes subjects aged six to twenty four months, suffering from haemorrhagic septicaemia. On the basis of clinical severity of the disease, they were randomly divided into two equal groups viz., A and B of twenty five animals each. Group A was treated with conventional treatment, while group B was infused with 7.5% NaCl solution in addition to conventional treatment.

Comparative Efficacy of Two Treatment Protocols

The comparative efficacy of two treatment protocols was assessed on the basis of analysis of different parameters as under;

4.1.1. Clinical Parameters

Survival rate

Each group was comprised of 25 buffaloes at the start of treatment. After treatment, higher mortality rate was observed in the buffaloes treated with conventional treatment protocol (group A), while group B showed significantly higher number of reviving animals (Table 2). The percent survival observed at t=36 h was 52 and 80 in group A and B, respectively. Group B differed significantly to group A from t=6 h to t=36 h (Table 3).

Severity index

Severity of disease varied in each animal. Animals were divided randomly in two groups on the basis of severity of the disease and a non-significant difference was observed in their mean values. After treatment, a remarkable decrease in severity index was observed in animals of group B treated with HSS along with ceftiofur and flunixin at each observational time and showed significant difference ($P < 0.05$) over groups A from
t=3 h to t=36 h. Group A also showed decrease in the severity index but the difference was non-significant up to t=6 h. At t=12 h, it decreased significantly ($P < 0.05$).

**Table 2. Number of animals survived from HS after treatment**

<table>
<thead>
<tr>
<th></th>
<th>Time after treatment (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td>25</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td>25</td>
</tr>
</tbody>
</table>

**Table 3. Survival rate (%) among two groups of animals suffering from HS after treatment**

<table>
<thead>
<tr>
<th></th>
<th>Time after treatment (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td>100</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td>100</td>
</tr>
</tbody>
</table>

* indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours).

**Table 4. Reduction in severity index of haemorrhagic septicaemia at different hours in groups after two treatment protocols**

<table>
<thead>
<tr>
<th>Observational Hours</th>
<th>Treatment allotted groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Group A</strong></td>
</tr>
<tr>
<td>Baseline</td>
<td>37.4 ± 3.2$^A$</td>
</tr>
<tr>
<td>1</td>
<td>33.2 ± 2.5$^{AB}$</td>
</tr>
<tr>
<td>3</td>
<td>30.8 ± 2.2$^B$</td>
</tr>
<tr>
<td>6</td>
<td>32.5 ± 1.7$^{AB}$</td>
</tr>
<tr>
<td>12</td>
<td>26.2 ± 2.3$^{BC}$</td>
</tr>
<tr>
<td>24</td>
<td>20.8 ± 1.2$^{C}$</td>
</tr>
<tr>
<td>36</td>
<td>17.6 ± 1.1$^{CD}$</td>
</tr>
</tbody>
</table>

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

High rise in body temperature was noted in all buffaloes suffering from haemorrhagic septicaemia. After institution of allocated treatments, a rapid fall in temperature was observed at t=1 h in both the groups which showed significant difference ($P < 0.05$) within groups from baseline values. Body temperature elevated significantly at t=3 h in both the groups which again decreased significantly ($P < 0.05$) at t=12 h but no significant difference between the two groups was observed at that time. At t=24 h, group B showed significant difference ($P < 0.05$) over group A (Fig-1). A non-significant difference ($P < 0.05$) was observed between two groups throughout the study period except at t=24 h. However, better trend toward recovery was observed in group B at each observational time. Both group attained normal values of body temperature (reference value: 38.5°C) within the study period.

Fig-1. Body temperature (°C) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).
Respiration rate

Buffaloes suffering from haemorrhagic septicaemia showed difficult breathing and decreased respiration rate was observed at baseline which differed non-significantly during the course of disease in all the animals of both the groups. After initiation of treatment, significant ($P < 0.05$) increase was noted within the groups followed by decreased respiration rate again at $t=6$ h. At $t=12$ h, a non-significant increase was observed in groups A and B and group B showed significant difference ($P < 0.05$) over group A. After that, both the groups showed increasing trend toward the normal (reference value: 15-30 breaths/min). At $t=36$ h, animals of group B showed normal respiration rate, while mean values were almost normal in group A (Fig-2).

Fig-2. Respiration rate (breaths/min) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftriaxone and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftriaxone and flunixin (group B). * 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 ($P > 0.05$).
Heart rate

Decreased heart rate was observed in buffaloes of both the groups before initiation of treatment and differed non-significantly. Rapid and significant increase was noted after treatment in both the groups but group B showed significant difference ($P < 0.05$) and heart rate was normal (reference value: 60-80 beats/min). After $t=1$ h, group A showed significant ($P < 0.05$) decrease in values again and these values continuously decreased up to $t=6$ h, while group B showed significant decrease only at $t=6$ h in mean values. At $t=12$ h, significant ($P < 0.05$) increase was observed again in heart rate of both groups. Group B showed significant difference ($P < 0.05$) over group A at each observational time throughout the study period after treatment (Fig-3). Animals of group B treated with hypertonic saline solution recovered normal values within $t=1$ h, while heart rate was almost normal in buffaloes of group A at $t=36$ h.

![Heart rate graph](image)

Fig-3. Heart rate (beats/min) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours). Means ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
4.1.2. Haematological Analysis

Haematocrit

Decreased values of haematocrit (Hct) were observed at baseline in both groups and differed non-significantly. A rapid increase in the mean values of Hct was observed in the animals of group B after administration of HSS and showed significant difference \((P < 0.05)\) over group A in which a mild increase was observed just after treatment. Haematocrit values in group B showed statistical significant \((P < 0.05)\) to group A at each observational time during the whole study. In group B, the values were near to normal (reference value: 30-35%) at \(t=12\) h and became normal at \(t=24\) h (Fig-4). On the other hand, values of Hct in group A did not become normal at \(t=36\) h. In group A, non-significant differences were observed after \(t=12\) h. But group B showed significant \((P < 0.05)\) increase in mean values of Hct up to \(t=24\) h and differed significantly \((P < 0.05)\) from group A.

Fig-4. Haematocrit (%) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 \((P > 0.05)\).
Haemoglobin concentration

Values for haemoglobin concentration (Hb concentration) in buffaloes suffering from HS observed below the reference values (14 g/dl). At baseline, non-significant difference was observed between two groups. After initiation of the respective treatments to the groups, increasing trend toward normal (reference value: 12-15 g/dL) was observed. Buffaloes treated with conventional treatment (group A) showed slower recovery of Hb concentration toward normal but recovery was rapid in animals treated with hypertonic saline solution (group B). Group B showed significant difference ($P < 0.05$) over group A at each observational time after institution of treatment (Fig-5). Group B recovered the Hb concentration significantly ($P < 0.05$) at each interval, while group A showed fluctuating trend and significant increase was observed at $t=3$ h and then it increased but non-significantly except at $t=36$ h where it showed statistical significant($P < 0.05$) difference.

Fig-5. Hb concentration (g/dL) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours). Means ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
4.1.3. Haemodynamic Parameters

Mean arterial pressure

At baseline, mean arterial pressure (MAP) decreased below 60 mm Hg in both groups that confirmed the development of septic shock during HS. Hypertonic saline infusion in buffaloes of group B increased MAP to normal (reference value: 78-88 mm Hg) at t=3 h and showed significant difference ($P < 0.05$) over group A, while a non-significant increase was observed in group A (Fig-6). Group B showed significant difference ($P < 0.05$) over group A at each observational time up to t=24 h. An increasing trend was observed in mean values of MAP in group A but failed to attain normal range within the study period. In group B, after achieving the normal value at t=3 h, a non-significant decline was noted and then it remained unchanged throughout the study period.

Fig-6. Mean arterial pressure (mm Hg) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).
**Central venous pressure**

At baseline, a severe decrease in the mean values of central venous pressure (CVP) was noted which started increase after initiation of treatment in both groups. Buffaloes treated with conventional treatment (group A) showed significant ($P < 0.05$) increase in the trend at $t=3$ h and this trend was observed up to $t=24$ h. In hypertonic saline treated buffaloes of group B, a rapid increase was observed just after institution of treatment and showed significant difference ($P < 0.05$) over group A at each observational time throughout the study period. Peak value was noted at $t=12$ h which was in the range of normal values (reference value: 10-20 mm Hg). Group A could not achieve normal values even at $t=36$ h (Fig-7).

![Central Venous Pressure](image.png)

Fig-7. Central venous pressure (mm Hg) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).
4.1.4. Blood Gas Analysis

Arterial oxygen

At baseline, partial pressure of arterial oxygen (PaO$_2$) decreased in all the animals of both groups suffering from HS. These values of PaO$_2$ were lowered to half of the reference values (80 mm Hg). After intravenous infusion of HSS, a rapid and significant ($P < 0.05$) increase in the PaO$_2$ mean values was noted in animals of group B. These values continuously rose up to $t=6$ h and showed significant difference ($P < 0.05$) but after $t=6$ h, unnoticeable difference was observed (Fig-8). On the other hand, group A showed a slow increasing trend and significant differed values at $t=3$ h up to $t=24$ h, but could not recover normal values (reference value: 80-92 mm Hg) within the study period. Group B showed significant difference ($P < 0.05$) to the group A throughout the study period except at $t=36$ h.

Fig-8. Partial pressure of arterial oxygen (mm Hg) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).
Venous carbon dioxide

Partial pressure of venous carbon dioxide (PvCO$_2$) increased during the disease in both groups. After treatment, PvCO$_2$ decreased significantly ($P < 0.05$) in animals of group B which were infused with HSS and showed significant difference ($P < 0.05$) to the group A from t=3 h to t=12 h. After t=12 h, there was non-significant difference between groups A and B (Fig-9). Buffaloes of group A showed decreasing trend in the PvCO$_2$ but this difference was non-significant up to t=12 h. At t=24 h, a significant ($P < 0.05$) decrease in the mean values of PvCO$_2$ was noted in group A and became non-significant from group B. At t=36 h, mean values of PvCO$_2$ in group A were near to the normal values (35-40 mm Hg), while group B recovered this value at t=12 h.

Fig-9. Partial pressure of venous carbon dioxide (mm Hg) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).
**Blood pH**

Before initiation of treatment, decreased values of venous blood pH were observed in buffaloes of both groups. After treating the animals with their allotted protocols, group A showed significant increase in the values of blood pH toward recovery up to $t=6\ h$. After $t=6\ h$, significant decrease in the values was observed which again increased significantly ($P < 0.05$) after $t=12\ h$. In group B, the mean values of blood pH significantly decreased between $t=1\ h$ and $t=3\ h$ after treatment and then rapidly increased ($P < 0.05$) toward normal. After initiation of treatment, group A showed significant difference ($P < 0.05$) over group B from $t=1\ h$ to $t=6\ h$ (Fig-10). But after $t=12\ h$, the picture became reverse and group B showed significant difference ($P < 0.05$) over group A and blood pH was in normal ranges (reference value: 7.35-7.45) at $t=24\ h$, whereas, in group A it was near to normal even at $t=36\ h$.

![Graph](Image)

Fig-10. Blood pH in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).
Bicarbonates

The values of bicarbonates (HCO$_3^-$) also decreased in the buffaloes suffering from HS. The trend after treatment was almost similar as observed in blood pH. In group A, slow increasing trend was observed in the values of bicarbonates toward recovery just after institution of the respective treatment up to $t=6$ h and then it transiently decreased at $t=12$ h followed by gradual increase. The values of bicarbonates initially decreased non-significantly after treatment in group B. After $t=6$ h, these values significantly ($P < 0.05$) increased rapidly and became normal (reference value: 22-30 mmol/L) at $t=24$ h (Fig-11), while treatment with conventional therapy in buffaloes of group A were unable to achieve normal values during the study period. In recovery of bicarbonates, group B showed significant difference ($P < 0.05$) over group A only at $t=24$ and $t=36$ h when it attained normal values.

Fig-11. Bicarbonates (mmol/L) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).
4.1.5. Serum Electrolytes

Serum sodium ions concentration

The increased concentration of serum sodium ions (Na⁺) were observed at baseline. After treatment, Na⁺ concentration gradually decreased and became normal (reference value; 132-140 mmol/L) within the study period in group A. Hypertonic saline infusion caused an increase in concentration of serum Na⁺ ions than the baseline in group B. At t=3 h of HSS infusion, sodium ions concentration attained its peak value (164.21± 7.92 mmol/L). Then it decreased toward normal significantly \((P < 0.05)\) and became normal at t=24 h (Fig-12). Group A showed significant difference \((P < 0.05)\) over group B after institution of the treatment up to t=6 h, and then non-significant difference was observed between both groups.

![Fig-12. Serum sodium ions concentration (mmol/L) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 \((P > 0.05)\).](image-url)
Serum chloride ions concentration

The concentration of chloride ions (Cl\(^-\)) were also increased in buffaloes of both groups during HS and differed non-significantly. After institution of treatment, a non-significant decrease toward normal was noted in group A. At t=12 h, the concentration of Cl\(^-\) ions became normal (reference value: 99-107 mmol/L) in group A, while HSS infusion increased the Cl\(^-\) concentration from the baseline in group B and this increasing trend was noted up to t=3 h. Then this concentration started decrease and became normal within the study period (Fig-13). Conventional treatment protocol (group A) showed significant difference (\(P < 0.05\)) over experimental protocol (group B) from t=1 h to t=6 h of the treatment, while group B was unable to show any significant difference over group A throughout the study period.

Fig-13. Serum chloride ions concentration (mmol/L) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 (\(P > 0.05\)).
**Serum potassium ions concentration**

At baseline, decreased concentration of serum potassium ions were noted in the buffaloes suffering from HS and differed non-significantly. After treating the animals with their allocated treatments, group A showed gradual and continuous increase toward normal (3.9-5.0 mmol/L) and showed significant difference \((P < 0.05)\) over group B at \(t=1\) h and \(t=3\) h. In group B, significant decrease was noted just after the infusion of HSS up to 3\(^{rd}\) hour and then the potassium concentration increased significantly and became normal within the study period (Fig-14). Group B showed significant difference \((P < 0.05)\) over group A at \(t=36\) h. Group A failed to recover the normal mean values of potassium ions within the study period while group B successfully recovered these values.

Fig-14. Serum potassium ions concentration (mmol/L) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 \((P > 0.05)\).
4.1.6. Serum Biochemical Profile

Total serum protein

Decreased values of total serum protein (TP) were observed at baseline during HS. After treatment, group A exhibited fluctuated trend, while group B showed increasing trend after t=3 h. In group A, the mean values of TP increased after treatment up to t=3 h, then it decreased significantly at t=6 h followed by an increase. A significant increase was observed at t=36 h. In group B, HSS infusion caused a non-significant decrease in the values of TP at t=1 h but it increased at t=3 h and rapidly increased up to t=12 h and recovered the normal values (reference value: 67-75 g/L). Group B showed significant difference ($P < 0.05$) over group A from t=6 h to t=36 h (Fig-15). The values of TP were normalized at t=12 h by HSS infusion in buffaloes of group B, while group A failed to achieve the normal range of TP.

Fig-15. Total serum protein (g/L) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours). Means ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
**Serum albumin**

The concentration of serum albumin (ALB) decreased than the normal (25-32 g/L) during haemorrhagic septicaemia in buffaloes. After institution of the treatment, group A showed significant increase followed by non-significant decrease. After t=12 h, the concentration of ALB again increased significantly and this increasing trend was observed till the end of the study. In group B, ALB concentration decreased significantly at t=1 h and then a rapid and significant increase ($P < 0.05$) was observed up to t=12 h. Hypertonic saline infusion to the buffaloes of group B recovered the normal range of ALB at t=12 h, while group A was unable to do so. Hence, group B showed significant difference over group A from t=6 h to t=36 h (Fig-16).

![Fig-16. Serum albumin (g/L) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).]
Serum globulin

Decreased values of serum globulin (GLB) were noted at baseline and differed non-significantly. After treatment, mean values of GLB decreased significantly in at t=3 h followed by an increase at t=12 h. Group B exhibited increasing trend at t=1 h and continuously increased up to t=6 h. After t=6 h, a non-significant decrease was observed in the mean values of GLB. Group B also showed significant difference ($P < 0.05$) over group A from t=6 h to t=36 h (Fig-17) and restored the normal values (reference value: 32-36 g/dL) within the study period but group A could not achieve the normal range up to t=36 h.

Fig-17. Serum globulin (g/L) in buffaloes (n=25 animals/group) suffering from haemorrhagic septicaemia and response to treatments with ceftiofur and flunixin (group A) and IV administered hypertonic saline solution in combination with ceftiofur and flunixin (group B). * 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 ($P > 0.05$).
Plate 5. A buffalo heifer five days after recovery from clinical pneumonic HS. Notice the deterioration of body condition in the wake of clinical disease.

Plate 6. A culture of *Pasteurella multocida* isolated on blood agar from a clinical case of pneumonic HS.
4.2. Evaluation of Hypertonic Saline Solution in Induced Calf Scour

A total of 24 colostrum-fed, male buffalo calves formed the basis of part of the present study. After adaptation period of 7 days, these calves were induced diarrhoea with the oral administration of *E. coli* broth culture. After 6 to 8 hours, the protocol of diarrhoea induction resulted in intense aqueous diarrhea in 100% of the animals with a 10% dehydration degree. Twelve hours after the onset of diarrhoea, the calves were treated with isotonic (group A) and hypertonic (group B) saline solutions in combination with ceftiofur HCl and flunixin meglumine. The results obtained were as under:

4.2.1. Clinical Parameters

**Survival Rate**

Neonatal diarrhoea was successfully induced in all calves. Each group was comprised of 12 buffalo calves before initiation of treatment. After treatment, mortality was observed in calves of both groups treated with isotonic (group A) and hypertonic (group B) saline solutions along with ceftiofur and flunixin. A non-significant difference of survival rate was observed between groups, although numbers of animals revived were greater in group B (Table 5). The percent survival at t=36 h were 75 and 83.3 in groups A and B, respectively (Table 6).

| Table 5. Number of induced-diarrhoeic calves survived after treatment with two treatment protocols |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Time after treatment (hours)                     |                  | 1               | 3               | 6               | 12              | 24              | 36              |
| Baseline                                        | Group A          | 12              | 11              | 11              | 10              | 10              | 09              | 09              |
|                                                 | Group B          | 12              | 11              | 11              | 10              | 10              | 10              | 10              |

<table>
<thead>
<tr>
<th>Table 6. Survival rate (%) of animals of two groups treated with two treatment protocols</th>
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<td>Baseline</td>
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<td>Group A</td>
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<td>Group B</td>
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**Body temperature**

Mean values of body temperature observed in calves of control and test groups prior to infection differed non-significantly. The protocol to induce diarrhoea increased body temperature significantly compared to their basal values in calves of both groups. After treatment, body temperature decreased significantly in both groups but group B showed significant difference ($P < 0.05$) over group A at $t=1$ h. After $t=3$ h, body temperature of calves increased in group B, while group A exhibited no change and showed significant difference ($P < 0.05$) to group B. After $t=6$ h, significant decrease was observed again in group B and matched with basal values (Fig-18). Group A also recovered the baseline values but values roused again at the end of experimental period, although these were in normal ranges.

![Graph showing body temperature changes over time](image)

**Fig-18.** Body temperature ($^{\circ}$C) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
**Respiration rate**

Non-significant differed values of respiration rate were observed in calves of both groups prior to infection. Induction of diarrhoea significantly decreased ($P < 0.05$) respiration rate to their basal values in all experimental calves. After treating the diarrhoic calves with their allocated protocols, respiration rate increased significantly in both the groups followed by significant decrease again. Calves of group A showed significant increasing trend ($P < 0.05$) than its counterpart after $t=3$ h but, in group B, these values continuously decreased non-significantly. Group B showed rapid increase ($P < 0.05$) in values of respiration rate after $t=6$ h and showed significant difference ($P < 0.05$) over group A at $t=24$ h (Fig-19). Both the groups recovered the baseline values of respiration rate within experimental period.

![Respiration rate graph](image-url)

**Fig-19.** Respiration rate (breaths/min) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Heart rate

The values of heart rate were non-significant differed in calves of control and experimental groups prior to infection. Increased heart rate was observed during diarrhoeic condition in calves of both groups. After institution of treatments, heart rate decreased ($P < 0.05$) in both groups. In group B, increasing trend was then observed in the values followed by sharp decrease toward baseline values (Fig-20). While group A showed continuous decrease up to $t=3$ h after treatment and showed significance differed ($P < 0.05$) values at this interval than group B followed by significant increase in its values. Both the groups recovered baseline values within observing time.

Fig-20. Heart rate (beats/min) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.2.2. Haematological Analysis

Haematocrit

The values of haematocrit (Hct) differed non-significantly between control and experimental groups before induction of scour to calves. Increased values of Hct were noted during diarrhoea. Infusion of fluids along with antibiotic and NSAID to the calves helped in decreasing the increased values of Hct significantly. Both the groups showed good recovery toward baseline but better recovery rate was observed in group B which was treated with hypertonic saline solution (Fig-21). The values of Hct slightly increased non-significantly at $t=3$ h but then again it started decrease and then continuously decreased to attain the normal value. The baseline values were recovered within 12 hours post-treatment in both groups.

Fig-21. Haematocrit (%) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
**Haemoglobin concentration**

Non-significant difference of haemoglobin (Hb) concentration between control and experimental groups were observed before induction of diarrhoea. The protocol to induce diarrhoea increased concentration of Hb in neonate calves. After administration of isotonic and hypertonic saline solutions to groups A and B, respectively, values of Hb concentration decreased significantly ($P < 0.05$). Both treatment protocols showed similar trend in recovering the Hb concentration toward normal (Fig-22). At $t=6$ h, there was non-significant increase in the values of Hb in group B, while same trend was observed in group A at $t=12$ h. The baseline values were recovered within experimentally time in both groups.

![Graph showing Hb concentration over time](image)

**Fig-22.** Hb concentration (g/dL) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.2.3. Haemodynamic Parameters

Mean arterial pressure

Mean values of mean arterial pressure (MAP) observed in calves of control and test groups prior to infection differed non-significantly. During diarrhoea, MAP decreased ($P < 0.05$) in both groups. Within first hour after treatment, it increased in both groups but group B showed significant increase ($P < 0.05$) and baseline values were achieved within 3 hours after infusion of HSS and showed significant difference ($P < 0.05$) over group A. At $t=6$ h, group B showed non-significant decrease in values of MAP which was transient and recovered within experimental period (Fig-23). Significant decrease in values of MAP was observed at $t=12$ h in group A. After $t=12$ h, the values increased ($P < 0.05$) and recovered near to baseline during study.

![Graph showing mean arterial pressure in buffalo calves](image)

Fig-23. Mean arterial pressure (mm Hg) in buffalo calves ($n=12$ animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Central venous pressure

Non-significant differed values of central venous pressure (CVP) were observed in calves of both groups prior to infection. Central venous pressure decreased significantly in all the calves induced with diarrhoea. After institution of treatment, significant increased was observed in both groups but group B which was treated with HSS recovered the basal values within 3 hours and showed significant difference ($P < 0.05$) to group A throughout the study except $t=6$ h, at which it showed significant fall in CVP and non-significant difference to group A (Fig-24). The values of CVP also increased significantly in group A and baseline value was recovered within 6 hours but then it decreased significantly and never returned back to baseline in experimental period.

Fig-24. Central venous pressure (mm Hg) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.2.5. Blood Gas Analysis

Arterial Oxygen

Partial pressure of arterial oxygen (PaO₂) was non-significant differed in calves of control and experimental groups prior to induction of infection. During infection (diarrhoea), significant decrease was observed in arterial oxygen pressure which increased significantly in both groups after treatment. Group B showed better recovery toward basal values and showed significant difference (\(P < 0.05\)) to group A at each observational time throughout the experimental period (Fig-25). The values of PaO₂ increased significantly up to 3 hours in group A after treatment. Then these values decreased followed by increase after \(t=12\) h. Group A was failed to achieve baseline values throughout the study, while group B almost recovered the basal values of PaO₂.

![Blood Gas Analysis Graph](image)

Fig-25. Partial pressure of arterial oxygen (mm Hg) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 (\(P > 0.05\)).
Venous carbon dioxide

Partial pressure of venous carbon dioxide (PvCO₂) differed non-significantly between control and experimental groups before induction of calf scour. Significant increase was noted in PvCO₂ during diarrhoea. After institution of treatment, significant decrease was noted within 1st hour in group B, while there was no change in the values of PvCO₂ in group A. After t=1 h, these values decreased in group A significantly, while continuous decrease was observed in group B and it showed significant difference (P < 0.05) to group A except t=12 h and t=24 h, where, sudden rise in venous CO₂ pressure was observed. Decreasing trend was also noted in group A toward baseline but failed to achieve it within experimental period, while HSS infusion to the calves of group B helped in achieving the basal values within 36 hours (Fig-26).

Fig-26. Partial pressure of venous carbon dioxide (mm Hg) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
**Blood pH**

Mean values of blood pH observed in calves of control and test groups prior to infection differed non-significantly. During diarrhoea, significant decrease \((P < 0.05)\) in its values was observed in both groups. After treatment, no improvement was observed in pH at \(t=1\) h in group B, while group A showed increase, however, it was non-significant. At \(t=3\) h, significant improved values of pH were observed in group B followed by decrease again. Same trend was noted in group A. At \(t=12\) h, group B showed significant increase \((P < 0.05)\). Both the groups were non-significant to each other and failed to achieve basal values throughout the study period (Fig-27).

![Blood pH in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 \((P > 0.05)\).](image-url)
**Bicarbonates**

The mean values of bicarbonates (HCO$_3^-$) observed in calves of control and experimental groups prior to induction of diarrhoea differed non-significantly. During diarrhoea, significant decrease ($P < 0.05$) in its values was observed in both groups. After treatment, significant increase was noted in values of HCO$_3^-$ in group A, while these values became worsen in 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-28). Both the groups recovered the values of HCO$_3^-$ near to baseline within experimental period.

![Fig-28. Bicarbonates (mmol/L) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).](image-url)
4.2.5. Serum Electrolytes

Serum sodium ions concentration

The concentration of sodium ions (Na\(^+\)) were non-significantly differed in both (control and test) groups prior to infection. Hyponatraemia was observed in calves submitted to the protocol to induce diarrhea. A significant increase ($P < 0.05$) in the sodium concentration was observed 3 hours after infusion of hypertonic saline solution, contrarily a non-significant difference was noted in group A which was treated with isotonic saline solution (Fig-29). The Na\(^+\) ions concentration decreased after $t=3$ h rapidly and matched that of basal values within experimental period in group B and it also showed significant difference ($P < 0.05$) to group A at $t=3$ h and $t=12$ h, while in group A, a sudden increase ($P < 0.05$) was observed at $t=24$ h and attained basal values.

Fig-29. Serum concentration of sodium ions (mmol/L) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Serum chloride ions concentration

The concentration of chloride ions (Cl\(^-\)) were non-significantly differed in both (control and test) groups prior to infection. During diarrhoea, there was hypochloremia in the calves of all groups submitted to the protocol to induce diarrhea (Fig-30). In the group treated with hypertonic saline, in contrast to the treatment with isotonic saline, chloride concentration increased significantly \((P < 0.05)\) after intravenous infusion. Serum chloride concentration attained peak level at \(t=3\) h. At \(t=6\) h, serum chloride concentration decreased \((P < 0.05)\), going back to the value at baseline. The concentration of chloride ions in calves treated with isotonic saline increased significantly \((P > 0.05)\) at \(t=3\) h. At \(t=24\) h, chloride concentration reached values similar \((P > 0.05)\) to those of the basal time (Fig-30).

Fig-30. Serum concentration of chloride ions (mmol/L) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 \((P > 0.05)\).
Serum potassium ions concentration

Non-significant difference was observed in the serum potassium (K⁺) ions concentration between both groups prior to infection. The protocol to induce diarrhoea decreased \((P < 0.05)\) serum concentration of potassium compared to their basal values. Serum potassium concentrations in calves treated with hypertonic and isotonic saline solutions presented different results during the experimental period (Fig-31). In the group treated with hypertonic saline (group B), potassium concentration first decreased followed by rapid increase and matched that of the baseline \((p>0.05)\) at \(t=36\) h. In the group treated with isotonic saline (group A), K⁺ concentration increased and significantly differed \((p<0.05)\) from group B at \(t=3\) h. However, after \(t=6\) h, the potassium value in the group treated with isotonic saline was similar to those of the hypertonic saline and basal values were recovered within 36 hours.

Fig-31. Serum concentration of potassium ions (mmol/L) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 \((P > 0.05)\).
4.2.6. Serum Biochemical Profile

Total serum protein

The concentration of serum protein differed non-significantly in calves of control and experimental groups prior to infection. In the calves submitted to protocol to induce diarrhoea, total serum protein (TP) decreased than the baseline. Significant increase was observed after administration of isotonic saline in animals of group A and hypertonic saline in calves of group B at t=3. At t=6, group B showed non-significant decrease. After t=6, significant increase was noted in group B which differed significantly from group A at t=24 and matched the basal values. Group A did not show any increase in serum protein after t=3 and were near to baseline values within 36 hours (Fig-32).

Fig-32. Total serum protein (g/L) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
Serum albumin

The concentration of serum albumin (ALB) differed non-significantly in calves of control and experimental groups prior to infection. During diarrhoea, serum albumin (ALB) decreased non-significantly. After institution of the treatment, group A showed significant increase followed by non-significant decrease. After t=12 h, the concentration of ALB again increased significantly and this increasing trend was observed till the end of the study. In group B, ALB concentration decreased significantly at t=1 h and then a rapid and significant increase ($P < 0.05$) was observed up to t=12 h. Hypertonic saline infusion to the buffaloes calves of group B recovered the normal range of ALB at t=12 h, while group A was unable to do so. Hence, group B showed significant difference over group A from t=6 h to t=36 h (Fig-33).

Fig-33. Serum albumin (g/L) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Serum globulin

The concentration of serum globulin (GLB) differed non-significantly in calves of control and experimental groups prior to infection. Decreased values of serum globulin (GLB) were noted during diarrhoea that differed non-significantly. After treatment, mean values of GLB decreased significantly in at t=3 h followed by an increase at t=12 h. Group B exhibited increasing trend at t=1 h and continuously increased up to t=6 h. After t=6 h, a non-significant decrease was observed in the mean values of GLB. Group B also showed significant difference ($P < 0.05$) over group A from t=6 h to t=36 h (Fig-17) and restored the normal values within the study period but group A could not achieve the normal range up to t=36 h.

Fig-34. Serum globulin (g/L) in buffalo calves (n=12 animals/group) with induced neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.3. Evaluation of Hypertonic Saline Solution in the Treatment of Clinical Conditions of Livestock Characterized by Hypovolaemia and/or Endotoxaemia

The clinical conditions of livestock included in the study were as follows:

A. Spontaneous calf scour: In this part of clinical study, 24 client-owned buffalo calves presented to the Veterinary Medical Teaching Hospital (VMTH), Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Calves were selected on the basis of following criteria: age < 25 days, neonatal diarrhoea (defined as soupy or watery consistency of the faeces), moderate to severe dehydration (skin tenting time > 3 but < 10 seconds, eyes slightly to markedly recessed into orbit), mild or no suckling reflex and no clinical symptoms of secondary organ diseases.

B. Diarrhoeic dehydrated goats: A total of 24 adult, female goats suffering from Peste des Petitis Ruminants (PPR) were admitted at VMTH of the department. The goats considered for the study had have severe dehydration due to diarrhoea that is a clinical symptom of PPR along with other characteristic signs such as high rise in body temperature (above 40 °C), coughing, sneezing and development of necrotic lesions in mouth extended over the entire mucosa (Radostits et al., 2007).

C. Diarrhoeic buffaloes: Adult buffaloes (n=24) suffering from diarrhoea were included in the study. Diarrhoea was defined as the voiding of fluid faeces that splashed when hitting the ground, with failure to form a firm, round buffalo pat.

The treatment protocol and evaluation parameters were same as in induced calf scour. The results obtained were as under.
4.3.1. Spontaneous Cases of Calf Scour in Buffalo Calves

4.3.1.1. Clinical Parameters

Survival rate

A total of 24 buffalo calves with neonatal diarrhoea were presented at VMTH and were randomly divided into two equal groups (A and B) comprised of 12 calves each. After treatment, mortality was observed in calves of both groups treated with isotonic (group A) and hypertonic (group B) saline solutions along with ceftiofur and flunixin. The number of animals revived in group B differed significantly ($P < 0.05$) from group A at t=24 h and t=36 h (Table 7). The percent survival observed at t=36 h was 58.3 and 75 in groups A and B, respectively. Group B differed significantly to group A at t=24 h and t=36 h (Table 8).

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</tr>
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<td>Group B</td>
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</tr>
</tbody>
</table>

$^*$ indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours).
Body temperature

Increased body temperature (39.73± 0.24 and 40.03± 0.23) was noted in calves during diarrhoea which differed non-significantly. After treatment, body temperature significantly decreased ($P < 0.05$) in both groups but group B showed significant difference ($P < 0.05$) to group A at $t=3$ h (Fig-35). At $t=6$ h, it increased again and matched to the values of group A and then non-significant difference between the two groups was observed throughout the study period. At $t=12$ h, both groups showed normal temperature (reference value; 38.5 $^\circ$C) which non-significantly increased at $t=36$ h, but these values were in normal ranges.

Fig-35. Body temperature ($^\circ$C) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Respiration rate

Increased respiration rate in diarrhoeic calves before the treatment was observed, which decreased after treatment, with significant difference ($P > 0.05$) between groups A and B at $t=1$ h. At $t=3$, significant decrease were noted in both groups treated with hypertonic and normal saline followed by increase in group A. At $t=6$ h, group B showed significant difference to group A followed by significant increase at $t=12$ h (Fig-36). After $t=6$ h, there was no significant difference between the groups throughout the experimental period with fluctuating values in both groups. The values at $t=36$ h were in range of normal values (reference value: 15-30 breaths/min) with non-significant difference between the groups.

Fig-36. Respiration rate (breaths/min) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
**Heart rate**

Diarrhoea in calves caused increase in heart rate. After infusion of hypertonic saline to calves of group B, heart rate increased significantly at t=1 h followed by significant decrease at t=3 h. In group A, just after infusion of normal saline, a sharp decrease was observed which showed significant difference to group B up to t=3 h (Fig-37). At t=6 h, heart rate increased significantly in group A, while it continuously decreased in group B which differed significantly to group A. At t=12 h, no change was observed in group B, while values decreased in group A significantly. After t=12 h, non-significant differences were observed between and within both groups and matched with the normal values (reference value: 60-80 beats/min).

![Fig-37. Heart rate (beats/min) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).](image-url)
4.3.1.2. Haematological Analysis

Haematocrit

The values of haematocrit (Hct) increased in diarrhoeic calves of both groups which decreased ($P < 0.05$) significantly after administration of hypertonic saline in calves of group B and showed significant difference to group A at $t=1$ h. Administration of normal saline to the calves of group A also decreased the values of Hct significantly at $t=3$ h followed by non-significant increase (Fig-38). Group B showed normal values (reference value: 30-35) of Hct at $t=6$ h which significantly differed ($P < 0.05$) from group A, while group A showed significant difference ($P < 0.05$) to group B at $t=24$ h, when it became normal. After $t=6$ h, Hct values significantly roused in group B but became normal again within the experimental period (Fig-38).

Fig-38. Haematocrit (%) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
Haemoglobin concentration

Concentration of haemoglobin (Hb) also increased in diarrhoea. After treatment, hypertonic saline administration in calves of group B lowered it significantly to normal (reference value: 10-15) within 3 hours and showed significant difference ($P < 0.05$) over group A. At $t=6$ h, Hb concentration roused significantly followed by a rapid decrease ($P < 0.05$) toward normal again with no change in further experimental period. Infusion of normal saline to calves of group A decreased Hb concentration significantly at $t=6$ h which increased at $t=12$ h followed by decrease ($P < 0.05$) to normal at $t=36$ h (Fig-39).

Fig-39. Hb concentration (g/dL) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.3.1.3. Haemodynamic Parameters

Mean arterial pressure

During diarrhoea, calves of both groups exhibited decreased values of mean arterial pressure (MAP) which differed non-significantly. Administration of hypertonic saline in calves of group B persuaded rapid increase in MAP at t=1 h and showed significant difference ($P < 0.05$) to group A which exhibited non-significant increase after administration of normal saline.

At t=3 h, values increased significantly in groups A and B and values became near to normal in group B (reference value: 100-120 mm Hg) followed by significant decrease in group B (Fig-40), while group A showed fluctuated trend with no significant change throughout the experimental period. Significant increase ($P < 0.05$) was observed in group B which matched to the normal values at t=12 h and differed significantly from group A.

Fig-40. Mean arterial pressure (mm Hg) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Central venous pressure

Diarrhoeic condition in calves caused a sharp decrease in central venous pressure (CVP) from the normal range (reference value: 10-20 mm Hg). Infusion of normal and hypertonic saline solutions to calves of groups A and B, respectively, increased CVP significantly but group B showed significant difference ($P < 0.05$) to group A at $t=3$ h where it became normal (Fig-41). Significant decrease was observed in both groups at $t=6$ h followed by significant increase at $t=12$ h and group B showed values within normal range, while near to normal in group A and it recovered normal values within 36 hours.

Fig-41. Central venous pressure (mm Hg) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.3.1.4. Blood Gas Analysis

Arterial oxygen

Decreased partial pressure of oxygen (PaO₂) was noted in diarrhoeic calves of both groups. Treatment of calves with hypertonic saline (group B) significantly increased the PaO₂ after infusion at t=1 h, while calves of group A showed significant increase at t=3 h. At t=12 h, group B differed significantly from group A and recovered the PaO₂ to normal (reference value: 80-92 mm Hg), while group A increased significantly at t=24 h which was near to normal but not normal. At t=36 h, PaO₂ decreased again in group A and group B differed significant to it (Fig-42).

Fig-42. Partial pressure of arterial oxygen (mm Hg) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
Venous carbon dioxide

Increased partial pressure of venous carbon dioxide (PvCO$_2$) was noted in calves with diarrhoea. After treatment, hypertonic saline infusion in calves of group B significantly decreased PvCO$_2$ at t=1 h, while group A showed non-significant decrease. At t=3 h, both groups showed significant decrease followed by non-significant increase at t=6 h. In group B, PvCO$_2$ decreased significantly again to match with the normal values (reference value: 35-40 mm Hg) at t=12 h (Fig-43) and differed significantly to group A which entered in normal range at t=24 h and became normal within experimental period.

Fig-43. Partial pressure of venous carbon dioxide (mm Hg) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
### Blood pH

Decreased values of venous blood pH were observed in diarrhoeic calves of both groups which increased significantly in group B and non-significantly in group A at t=1 h. Group B showed significant increasing trend up to t=3 h to became near to the normal values (reference value) and differed significantly from group A from t=6 h to t=24 h. In group A, pH values decreased at t=6 h followed by slow and non-significant increase up to t=24 h. At t=36 h, values of blood pH were near to normal (reference value: 7.35-7.45), while group B achieved these values at t=12 h (Fig-44).

![Blood pH](image)

**Fig-44.** Blood pH in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Bicarbonates

The values for bicarbonates (HCO$_3^-$) became lower than the normal (reference value: 22-30) in diarrhoeic calves. After treatment, group A showed continuous increase in values of HCO$_3^-$ and recovered the normal values within experimental period. In group B, values of HCO$_3^-$ increased significantly up to t=12 h and showed significant difference to group A at t=3 h and t=12 h. Group B recovered normal values within 3 hours after infusion of hypertonic saline solution to calves. After t=12 h, decreasing trend was observed in group B but values were in normal ranges (Fig-45).

Fig-45. Bicarbonates (mmol/L) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.3.1.5. Serum Electrolytes

Serum sodium ions concentration

Hyponatraemia was observed in calves with neonatal diarrhoea. After infusion of hypertonic saline solution, a rapid and significant increase \((P < 0.05)\) was noted in calves of group B up to \(t=6\) h. Then decreasing trend was observed and concentration of serum sodium became normal (reference value: 132-140 mmol/L) within 24 hours in group B. Group A showed increasing trend after administration of normal saline solution to the calves. The values for serum sodium in group A matched with the normal within 24 hours. In group B, the serum sodium concentration crossed the limit of normal range in calves which was transient (Fig-46).

Fig-46. Serum concentration of sodium ions (mmol/L) in buffalo calves \((n=12\) animals/group\) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 \((P > 0.05)\).
Serum chloride ions concentration

The concentration of serum chloride ions dropped during neonatal diarrhoea. After infusion of normal and hypertonic saline solutions to the calves of groups A and B, respectively, increasing trends were observed in both groups but it was significant (P < 0.05) in group B. In this group, chloride concentration crossed the normal values (reference value: 99-107 mmol/L) within 3 hours (Fig-47) but transient and dropped after 3 hours significantly. Group A recovered the normal values of chloride ions at t=12 h which showed significant difference (P < 0.05) over group B. The chloride concentration increased in group B after t=12 h and became normal within experimental period (Fig-47).

Fig-47. Serum concentration of chloride ions (mmol/L) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
Serum potassium ions concentration

Increased serum potassium concentration was observed during neonatal diarrhoea in calves. Infusion of hypertonic saline to the calves of group B decreased potassium concentration more rapidly than the group A and showed significant difference over group A at t=6 h. In group A, significant decrease was noted at t=3 h followed by increased at t=6 h. After 6th hour, potassium concentration decreased and became normal (reference value: 3.9-5.0 mmol/L) within experimental period. Group B showed normal values and also significant difference to group A at t=24 h (Fig-48).

Fig-48. Serum concentration of potassium ions (mmol/L) in buffalo calves (n=12 animals/group) with neonatal diarrhoea and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
4.3.2. Evaluation of Hypertonic Saline Solution in the Treatment of Diarrhoeic Dehydrated Goats

4.3.2.1. Clinical Parameters

Survival rate

A total of 24 goats with diarrhoea were presented at VMTH and were randomly divided into two equal groups (A and B) comprised of 12 animals each. After treatment, mortality was observed in goats of both groups treated with isotonic (group A) and hypertonic (group B) saline solutions along with ceftiofur and flunixin. The number of animals revived in group B differed significantly ($P < 0.05$) from group A (Table 9). The percent survival observed at $t=36$ h was 50 and 75 in groups A and B, respectively. Group B showed significant difference ($P < 0.05$) over group A from $t=12$ h to $t=36$ h (Table 10).

Table 9. Number of goats revived after treatment with two treatment protocols

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Table 10. Survival rate (%) of animals of two groups treated with two treatment protocols

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* indicates significant difference ($P > 0.05$) between Group A and Group B at that time (hours).
**Body temperature**

Increased body temperature than the normal (reference value: 39.4 °C) was observed in diarrhoeic dehydrated condition of goats. Significant decrease (P < 0.05) was noted in both groups after treatment up to t=3 h. Body temperature increased again at t=6 h significantly followed by lowering and entered in normal range at t=12 h in both groups. At t=24 h, group A showed non-significant increase in temperature, while significant decrease was observed in group B and temperature became normal which differed significantly (P < 0.05) from group A. Group A also showed normal temperature at t=36 h (Fig-49).

![Fig-49](image)

**Fig-49.** Body temperature (°C) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
Respiration rate

Decreased respiration rate was observed in diarrhoeic dehydrated goats which non-significantly increased after treatment. However, group B showed better trend toward recovery than group A and differed significantly \((P < 0.05)\) from it at \(t=24\) h (Fig-50). Both groups achieved the normal values (reference value: 15-30 breaths/min) of respiration rate within 12 hours of treatment but group A followed a non-significant decrease after \(t=24\) h, however, obeyed the normal range.

Fig-50. Respiration rate (breaths/min) in dehydrated diarrhoeic goats \((n=12\) animals/group\) and response to treatments with IV administered isotonic (group A) and hypertonic (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 \((P > 0.05)\).
Heart rate

During diarrhoea, decreased heart rate was observed in goats. After treatment, it increased in both groups significantly at t=3 h. At t=6h, heart rate decreased in group A but increased in group B which showed significant difference ($P < 0.05$) to its counterpart. Reverse situation was observed in both groups at t=12 h followed by increase in heart rate in group B which showed significant difference ($P < 0.05$) to group A at t=24 h. Group B recovered the normal values (reference value: 70-90 beats/min) within 6 hours after treatment, while group A showed near to normal at t=36 h (Fig-51).

Fig-51. Heart rate (beats/min) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.3.2.2. Haematological Analysis

**Haematocrit**

Increased haematocrit (Hct) values were observed in diarrhoeic dehydrated goats which decreased significantly toward normal at t=3 h in both groups followed by increase. Group B showed significant difference ($P < 0.05$) over group A at t=6. Both groups decreased Hct values at t=12 h but group B showed significant decrease and became normal (reference value: 30-40%), hence, differed significantly again from group A. Group A recovered normal Hct values at t=24 h which again roused but remained within range of normal values (Fig-52).

![Graph showing haematocrit values over time](image-url)

**Fig-52.** Haematocrit (%) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
**Haemoglobin concentration**

The concentration of haemoglobin (Hb) increased during dehydrated diarrhoea. After treatment, significant decrease was observed at t=3 h followed by significant increase. Group B showed significant difference \( (P < 0.05) \) over group A at t=12 h but reverse situation was noted at t=24 h. Group B attained normal ranges (reference value: 12-16 g/dL) of Hb concentration on alternate observation interval started from t= 3 h, while group A showed normal concentration of Hb at t=24 h which again increased \( (P < 0.05) \) at the end of study (Fig-53).

Fig-53. Hb concentration (g/dL) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 \( (P > 0.05) \).
4.3.2.3. Haemodynamic Parameters

Mean arterial pressure

During diarrhoea, the mean arterial pressure (MAP) decreased (82 ± 13.68 and 80 ± 15.13). Significant increase was observed in MAP after administration of isotonic and hypertonic saline solutions to the goats of group A and B, respectively, but recovery was better in group B and it was near to normal at t=3 h which differed significantly from group A (Fig-54). At t=6 h, MAP decreased significantly in both groups and group B showed significant difference over group A. Both groups increased MAP to normal (reference value: 100-110 mm Hg) at t=12 h which slightly decreased at t=24 h followed by significant increase at t=36 h and became normal again in both groups. Although, MAP became normal in both groups but group B showed better trend throughout the experimental period after infusion of hypertonic saline solution (Fig-54).

Fig-54. Mean arterial pressure (mm Hg) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
Central venous pressure

Decreased values were observed in diarrhoeic goats which increased after treatment. At t=3 h, both groups showed significant increase than the basal values but values became normal (reference value) in group B which differed significantly ($P < 0.05$) from group A. The values of CVP decreased in both groups but significantly in group B at t=6 h followed by marked increase. Group B recovered values in a better way and showed significant difference ($P < 0.05$) over group A (Fig-55). At t=24 h, group B recovered normal values (reference value: 16-30 mm Hg) of CVP but these were near to normal in group A and were normal at t=36 h.

Fig-55. Central venous pressure (mm Hg) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
**4.3.2.4. Blood Gas Analysis**

**Arterial Oxygen**

The partial pressure of arterial oxygen (PaO\(_2\)) decreased during diarrhoeic condition in goats of groups A and B to 49.67 ± 8.62 and 46.67 ± 10.69, respectively. After treatment, it increased significantly in group B and non-significantly in group A at t=1 h. Group A showed significant increase in PaO\(_2\) than the basal values at t=3 h but group B increased the values to normal (reference value: 80-94 mm Hg) and differed significantly from group A (Fig-56). At t=6 h, PaO\(_2\) decreased in group B but significantly increased near to normal in group A followed by marked decrease but increase in group B. The values for PaO\(_2\) became normal in group B at t=12 h and showed significant difference to group A in which PaO\(_2\) markedly decreased. Group A increased the values near to normal within experimental period (Fig-56) but group B recovered normal PaO\(_2\) from t=3 h and maintained it throughout the study period except t=6 h.

Fig-56. Partial pressure of arterial oxygen (mm Hg) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
Venous carbon dioxide

The partial pressure of carbon dioxide (PvCO₂) increased during diarrhoea in goats of control and experimental groups. After treatment with their allotted protocols, significant decrease was noted in hypertonic saline treated goats, while non-significant decrease was observed in animals of group A which was treated with normal saline solution at t=1 h. Significant decrease was observed in groups A and B at t=3 h, after which PvCO₂ became near to normal (reference value: 38-45 mm Hg) in former, while normal in later group (Fig-57). Group B followed a non-significant increase at t=6 h which decreased to normal again at t=12 h and showed significant difference (P < 0.05) over group A in which PvCO₂ increased at the same observation time. Group A also normalized the values of PvCO₂ at t=24 h.

Fig-57. Partial pressure of venous carbon dioxide (mm Hg) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
**Blood pH**

The values for blood pH became lower than the normal (reference value: 7.32-7.44) in goats during diarrhoeic conditions. After institution of treatments, group A showed recovery toward normal rapidly up to t=6 h and differed significantly from its counterpart. Blood pH decreased in goats of group B at t=1 h which then increased at t=3 h to the basal values followed by non-significant decrease again at t=6 h. After t=6 h, group B recovered blood pH rapidly and values became normal within t=24 h and showed significant difference (P < 0.05) over group A. The blood pH transiently became normal at t=6 h which then decreased and remained below the normal for 24 hours but normalized within experimental period (Fig-58).

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**Fig-58.** Blood pH in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
**Bicarbonates**

Decreased values of bicarbonates (HCO₃⁻) were observed in goats during diarrhoea. After treatment, HCO₃⁻ increased in group A but decreased in group B at t=1 h. After that, a rapid increase (P < 0.05) was observed in group B followed by decrease at t=6 h. Group A showed significant difference (P < 0.05) over group B at t=6 h and values became near to normal (reference value: 24-30 mmol/L) which then decreased. Group B increased values of HCO₃⁻ significantly after t=6 h and became normal at t=24 h which differed significantly from group A. At t=36 h, group A also recovered the normal values (Fig-59).

Fig-59. Bicarbonates (mmol/L) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
4.3.2.5. Serum Electrolytes

Serum sodium ions concentration

The concentration of serum sodium decreased during diarrhoea in goats of both groups A and B. Administration of hypertonic saline solution to the animals of group B increased sodium concentration significantly up to t=3 h which differed significantly from group A from t=3 to t=12 h. The highest concentration of sodium (151.0 ± 11.37) was achieved at t=3 h which was over than the normal values (reference value: 130-146 mmol/L) but after that it rapidly decreased and became normal within 24 hours. On the other hand, normal saline administration to the goats of group A increased sodium concentration slowly and was near to normal within 3 hours but decreased to the baseline again at t=6 h. Then concentration increased again and became near to normal within t=24 h but never became normal throughout the study period (Fig-60).

Fig-60. Serum concentration of sodium ions (mmol/L) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Serum chloride ions concentration

The concentration of chloride (Cl\(^{-}\)) ions decreased than the normal (reference value: 95-110 mmol/L) in diarrhoeic goats of control and test groups. After administration of normal saline solution to the animals of group A, Cl\(^{-}\) concentration increased near to normal within 3 hours but then it decreased at t=6 h. The concentration of Cl\(^{-}\) again increased near to normal at t=12 h and never became normal within experimental period. Administration of hypertonic saline to the goats of group B increased Cl\(^{-}\) concentration significantly up to t=3 h which differed significantly from group A and then it decreased rapidly to normal within 24 hours (Fig-61).

Fig-61. Serum concentration of chloride ions (mmol/L) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (\(P > 0.05\)).
**Serum potassium ions concentration**

Increased concentration of potassium ions were observed in all goats suffering from diarrhoea. After administration of normal saline to the animals of group A and hypertonic saline to the goats of group B, potassium concentration decreased rapidly and became normal (reference value: 4.8-6.2 mmol/L) at t=3 h. Increased concentration was noted in group at t=6 h but no significant change was observed in group B which showed significant difference ($P < 0.05$) over group A. At t=12 h, mean values of potassium ions decreased and became normal again in group A. No significant change was observed in both groups at t=12 h and onward and potassium concentration remained normal during remaining study period (Fig-62).

![Graph showing serum potassium concentration over time](image)

**Fig-62.** Serum concentration of potassium ions (mmol/L) in dehydrated diarrhoeic goats (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.3.3. Evaluation of Hypertonic Saline Solution in Diarrhoeic Buffaloes

4.3.3.1. Clinical Parameters

Body temperature

A mild increase in body temperature was noted during diarrhoea in buffaloes of control and test groups. A continuous reduction in the body temperature was noted after treatment in both groups and it became normal (reference value: 38.5 °C) at t=3 h. At t=6 h, a marked increase was observed in both groups again. Group B showed decrease in body temperature again to normal at t=12 h and differed significantly from group A. In group A, it remained higher at t=12 h and then decreased to normal at t=24 h. Then temperature remained normal throughout study period in both groups (Fig-63).

![Graph showing body temperature changes](image)

Fig-63. Body temperature (°C) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
**Respiration rate**

A moderate increased respiration rate than the normal (reference value: 15-30 breaths/min) was observed in diarrhoeic buffaloes. Respiration rate increased in buffaloes of group B treated with hypertonic saline solution at t=1 h which decreased at t=3 h, while group A showed decrease and significant difference ($P < 0.05$) over group B at t=1 h. A non-significant increasing trend was also observed in group A at t=6 h followed by a decrease toward normal. Both groups showed normal respiration at t=12 h after respective treatment (Fig-64).

![Graph](image-url)

**Fig-64.** Respiration rate (breaths/min) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
Heart rate

During diarrhoea in buffaloes, heart rate increased than the normal values (reference value: 60-80 beats/min). After institution of the treatments, it decreased in group A and showed significant difference ($P < 0.05$) over group B in which heart rate increased at $t=1$ h and $t=3$ h. A rapid decrease ($P < 0.05$) was then observed in group B at $t=6$ h and mean values became normal at $t=12$ h which then increased but remained within the normal values. Group A also showed similar trend after $t=6$ h (Fig-65).

Fig-65. Heart rate (beats/min) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
4.3.3.2. Haematological Analysis

**Haematocrit**

Increased values of haematocrit (Hct) were observed during diarrhoea in buffaloes. After administration of allocated treatments, Hct values decreased in both group significantly and became normal (reference value: 30-35%) at t=3 h. Group A showed increase in its Hct values after t=6 h and exceeded normal values but a non-significant change was observed in group B and showed normal values during the rest of study period (Fig-66).

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**Fig-66.** Haematocrit (%) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
**Haemoglobin concentration**

The concentration of haemoglobin (Hb) increased during diarrhoea. After treatment, significant decrease was observed at t=1 h and t=6 h in groups B and A, respectively. Group B showed normal Hb concentration (reference value: 12-15 g/dL) within 3 hours which increased non-significantly at t=6 h. Group A normal values of Hb at t=6 h followed by non-significant increase at t=12 h (Fig-67). Both the groups A and B recovered normal values at t=24 h and t=12 h, respectively.

![Fig-67. Hb concentration (g/dL) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).](image-url)
4.3.3.3. Blood Gas Analysis

Venous Oxygen

Decreased pressure of venous oxygen (PvO₂) was observed during diarrhoea in buffaloes of both groups. Administration of hypertonic saline to the buffaloes of group B increased PvO₂ significantly to normal (reference value: 30-40) within 3 hours and showed significant difference over group A. At t=6 h, a non-significant decrease was observed in the PvO₂ in group B which was followed by a rapid and significant increase at t=12 h. Group B showed significant difference over group A at t=24 h and then decreased non-significantly but values were within normal range (Fig-68). Normal saline infusion to the animals of group A increased PvO₂ at each observation time and attained the normal values at t=12 h but decreasing trend was noted later on and PvO₂ decreased at the end of experimental period and values were below the normal range (Fig-68).

Fig-68. Partial pressure of venous oxygen (mm Hg) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
Venous carbon dioxide

Increased pressure of venous carbon dioxide (PvCO₂) was observed in diarrhoeic buffaloes. After administration of respective treatments, group A showed decrease in carbon dioxide pressure toward normal (reference value: 35-40 mm Hg) but better recovery was noted in group B and it significantly lowered the values of PvCO₂ to normal within 3 hours. At t=6 h, increased PvCO₂ was noted in both groups which decreased at next observation interval (t=12 h) and both groups showed normal values. No further change in PvCO₂ was observed in group B for the rest of the study period, while a non-significant increase was observed in group A which crossed the normal range of PvCO₂ (Fig-69).

Fig-69. Partial pressure of venous carbon dioxide (mm Hg) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 (P > 0.05).
Blood pH

The values of blood pH were decreased in buffaloes suffering from diarrhoea. After institution of treatment, blood pH increased rapidly to normal (reference value: 7.35-7.45) in group A, but after t=6 h, it decreased below the normal range at t=12 h. At t=24 h, the values of pH became normal again in group A and remained normal up to the end of study. In group B, the values of pH increased but trend was slow up to t=6 h. After t=6 h, it increased rapidly and became normal within 12 hours and then remained normal during the experimental period (Fig-70).

Fig-70. Blood pH in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
**Bicarbonates**

The values of bicarbonates decreased in diarrhoeic buffaloes. After treatment, significant increase was observed in both groups at $t=3$ h. Group B showed non-significant decrease at $t=6$ h followed by rapid increase and bicarbonates became normal (reference value: 22-30 mmol/L) at $t=12$ h which showed significant difference over group A. The values decreased at $t=36$ h non-significantly in group B but these were in normal ranges. Group A showed decreased values of bicarbonates at $t=12$ h which then increased and became normal. At $t=36$ h, a non-significant decrease was observed in bicarbonates but remained within normal (Fig-71).

![Fig-71](image-url)

Fig-71. Bicarbonates (mmol/L) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
4.3.3.4. Serum Electrolytes

Serum sodium ions concentration

A mild decrease than the normal (reference value) values was observed during diarrhoea in buffaloes of control and test groups. After infusion of hypertonic saline to the buffaloes of group B, a rapid increase in sodium concentration was observed and became higher than the normal values (reference value: 132-140 mmol/L) which remained higher for 3 hours when its peak values were obtained (160.75 ± 9.0). After t=3 h, a significant decrease was observed in sodium concentration and it became normal within 24 hours (Fig-72). Group A showed non-significant increasing trend toward normal and sodium concentration became normal within 6 hours which then decreased non-significantly before t=36 h but remained normal.

Fig-72. Serum concentration of sodium ions (mmol/L) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
**Serum chloride ions concentration**

The concentration of chloride decreased in diarrhoeic buffaloes. After administration of hypertonic saline to the buffaloes of group B, a rapid increase was noted and concentration became beyond the normal ranges (reference value: 99-107 mmol/L) within 3 hours. After t=3 h, chloride concentration decreased rapidly and became normal within 12 hours. At t=24 h, it decreased below the normal but then increased to normal again at t=36 h (Fig-73). Group A showed increase in the chloride concentration toward normal and the concentration became normal within 6 hours and remained normal throughout the study period with non-significant change.

Fig-73. Serum concentration of chloride ions (mmol/L) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($\pm$ SE) sharing similar letters within a group are statistically non-significant ($P > 0.05$).
**Serum potassium ions concentration**

Decreased concentration of serum potassium ions were observed in all buffaloes during diarrhoea. Administration of normal saline to the buffaloes of group A increased potassium concentration rapidly and showed significant difference over group B at t=1 h. Group B showed mild and non-significant increase at t=1 h and then it increased rapidly and showed greater potassium concentration at t=6 h. Both groups recovered normal (reference value: 3.9-5.0) within 6 hours and then remained normal throughout the experimental period with non-significant change (Fig-74).

**Fig-74.** Serum concentration of potassium ions (mmol/L) in diarrhoeic buffaloes (n=12 animals/group) and response to treatments with IV administered isotonic (group A) and hypertonic (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 ($P > 0.05$).
5.1. Evaluation of Hypertonic Saline Solution in the Treatment of Haemorrhagic Septicaemia

Haemorrhagic septicaemia, the number one killer disease of buffaloes (*Bubalis bubalis*) in Pakistan, is associated with *Pasteurella multocida* serotype B:2, a gram-negative bacterium (Carter and De Alwis, 1989). Consideration of pathophysiological features of this fatal disease could help in constructing the treatment protocol:

(a) The disease is associated with a gram-negative bacterium. As such the antibiotic administered must be specifically effective against gram-negative organism (Raza *et al*., 2000).

(b) The gram-negative bacteria secrete endotoxins in the blood which are responsible for all manifestations of the disease (Horadagoda *et al*., 2001). These endotoxins trigger arachidonic acid metabolites resulting in the production of prostaglandins and leukotrienes. These mediators would ensue moderate to severe pyrexia which is a hallmark of this disease. So, measures should be adapted to lower the elevated body temperature such as water hosing on the head before initiation of treatment and use of selective inhibitors of arachidonic acid metabolism i.e. non-steroidal anti-inflammatory drugs (NSAIDs) e.g. flunixin meglumine (Conlon, 1988; Raza *et al*., 2000).

(c) Endotoxins profoundly affects circulatory system including myocardial depression, pronounced vasodilation and alterations in the endothelial barrier which result in hypovolaemia and decreased cardiac output. It is, therefore, of great importance to maintain an adequate cardiac output and blood pressure through restoration of intravascular volume. Thus, in addition to antibiotic therapy, fluid administration plays a pivotal role in the management of sepsis and septic shock (Gow *et al*., 1998; Somell *et al*., 2005). Restoring a normal cardiac output requires intravascular replacement of fluid...
losses, compensation for venous pooling of blood and a sufficiently high left ventricular filling pressure to compensate for decreased ventricular contractility during sepsis (Parker et al., 1984; Parrillo et al., 1990).

The baseline data obtained during the course of haemorrhagic septicaemia substantiated the fact that the buffaloes studied had clinical, haematological and biochemical evidence of endotoxaemia. In the present study, regarding to aforementioned pathophysiological features of HS and importance of fluid therapy in the resuscitation of septic shock patients, a treatment protocol was constructed that animals should be treated with bolus administration of hypertonic saline solution in addition to ceftiofur hydrochloride (a third generation cephalosporin) and flunixin meglumine (a non-steroidal ant-inflammatory drug). So, this study was designed in this manner to mimic potential clinical use of hypertonic saline (7.5% NaCl) solution as an adjunct to antibiotic therapy. Three mechanisms have been proposed to explain beneficial clinical responses to rapid IV administration of HSS. The first hypothesis proposes that HSS causes rapid redistribution of body fluid from the intracellular to intravascular and extracellular fluid compartments, which enhances circulating blood volume and tissue perfusion (Bertone et al., 1990; Tyler et al., 1994). The second hypothesis states that hypertonic fluids exert a vagal-mediated inotropic effect on the heart (Lopes et al., 1981; Luypaert et al., 1986; Schmall et al., 1990). The third hypothesis proposes that the clinical response to HSS is a function of altered peripheral vascular resistance (Constable et al., 1991a).

The primary findings of this study are the increased survival rate in HSS treated animals of group B. The percent survival among animals treated with protocol A and B was 52 and 80, respectively. Difference in recovery rate between these protocols was highly significant. The higher recovery rate of 80% in group B than 52% in group A was attributed to the addition of hypertonic saline solution in the former treatment protocol. These findings confirmed the reports by Raza et al. (2000). They also reported survival rates of 85% in field cases of haemorrhagic septicaemia treated with norfloxacin, diclofenec sodium in combination with a circulatory stimulant Novacoc-Forte.

In the present study, a decrease in disease severity index was recorded in both groups after treatment but the buffaloes of group B showed significant difference over its
counterpart which are partially in line with the results of Raza et al. (2000). Reduction in severity index is presented in Table 4. This study revealed that the buffaloes treated with hypertonic saline solution in combination with ceftiofur and flunixin showed higher survival rate due to reduction in severity index.

Moderate to severe pyrexia was observed in all buffaloes which is the first clinical sign for the recognition of HS. Water hosing for 15 minutes effectively lowered body temperature before initiation of the treatment. Non-steroidal anti-inflammatory agent named flunixin meglumine was used in the study. Flunixin meglumine is responsible to diminish the effects of arachidonic acid metabolites which culminate in decreased level of prostaglandins and leukotrienes and ultimately lowers the body temperature (Plumb, 1999). In the present study, this drug helped in achieving the target of exerting the beneficial influence on the pathophysiologic consequences of endotoxaemia and lowering the elevated body temperature of the buffaloes suffering from HS. These results are in line of the previous studies (Verhoef et al., 1986; Anderson and Hunt, 1989; Yazar et al., 2007). The respiration rates in both groups were below the normal level; additionally, wheezing and increased breath sounds were detected during thoracic auscultation and grossly in some severe cases clearly indicated lung injury. Administration of hypertonic saline solution not only increased the respiration rate, but also diminished the lung sounds. A lung-protective, anti-inflammatory effect of HSS has been indicated to diminish the lung injury by reduction in neutrophils sequestration in the lungs after haemorrhagic shock (Lopes et al., 1981; Angle et al., 1998; Rizoli et al., 1998; Rizoli et al., 2006). The other advantage of HSS is the increase plasma osmolarity, therefore, the risk of oedema in lungs and other tissues may be brought down to a minimum (Constable et al., 1996; Senturk, 2003; Somell et al., 2007).

Heart rate decreased during septicaemic conditions in buffaloes of both groups. These results have already been predicted as in situation when plasma volume decreases (Lopes et al., 1986; Dubick et al., 1995; Gow et al., 1998). Reduced plasma volume resulted in decreased cardiac output escorted weak and declined heart rate (Rocha e Silva et al., 1986; Schmall et al., 1990; Constable et al., 1995). The group treated with hypertonic saline solution showed significant increase in heart rate compared to basal values and normalized it within an hour and then remained in the normal range
throughout the study period. The factor for increased heart rate in group B may be a result of the effect of HSS in decreasing the oedema in endothelial cells in the blood vessels, pre-capillary dilatation and decreasing in vascular resistance (Mazzoni et al., 1990; Corso et al., 1998). A rapid increase of serum osmolality is the other factor responsible for systemic arterial vasodilation, promoting immediate expansion of plasma volume and activation of cardiovascular reflex mechanisms (Velasco et al., 1980; Constable et al., 1995; Cambier et al., 1997; Constable, 1999). However, the beneficial effect in afterload was transient, lasting for 3 hours. Our findings differed from previous studies (Constable et al., 1995) in which these transient effects were of few minutes, probably due to specie difference.

Haemoglobin (Hb) concentration and haematocrit (Hct) are the indicators of total cells including erythrocytes, leukocytes and platelets. Any increase or decrease in values of both parameters showed the signs of dehydration and hypovolaemia and/or endotoxaemia, respectively (Reece, 1997). In the present study, a decreased value of both Hb and Hct clearly indicated reduction in the number of cells and is attributable to a progressive decrease in plasma volume. In group B, HSS rapidly increased Hct and Hb concentration values and played a pivotal role in restoring plasma volume and also increased osmosis and promoted movement of endogenous fluid from the extravascular to intravascular space (Constable et al., 1991b; Tyler et al., 1994). As no fluid was administered in animals of group A, so, increase Hct and Hb concentration values remained below the normal which ultimately resulted in decreased plasma volume. These observations strongly recommend that fluid administration is the basic tenet to resuscitate the patients from septic shock.

Endotoxaemia caused a substantial reduction in MAP and CVP, and additional loss of perfusion pressure due to systemic vasodilatation is not favourable in shock, and could in a longer perspective induce multiple organ dysfunction (MOD). This response is thought to be secondary to reduced cardiac contractility, decreased venous return and decreased cardiac output (Velasco et al., 1980; Kramer et al., 1986; Kreimeier et al., 1991). Our treatment group with the combination of ceftiofur HCl, flunixin meglumine and HSS had the most pronounced increase in MAP and CVP compared with control group (without supportive therapy to the animals in this group). Principally, the rise in
serum osmolarity is thought to cause the major effects on MAP and CVP by osmotic extraction of water from the interstitium and expansion of the intravascular volume (Velasco et al., 1980; Constable et al., 1991a; Rocha e Silva and Pol de Figueiredo, 2005). The circulation seems to improve, however, only if the hyperosmotic infusion is given proximally to the pulmonary circulation (Lopes et al., 1981). Other explanations were that this phenomenon may have because by the vagus nerve reflex and expanding plasma volume (Velasco et al., 1980; Rocha e Silva et al., 1987). Results of this study indicated that rapid IV administration of HSS to septicemic buffaloes induced an immediate, beneficial and sustained haemodynamic improvement. This finding indicates that the haemodynamic response to HSS dependant on the quantity of sodium ions administered and also influenced by the tonicity of the solution and availability of free water accompanying the sodium ions (Constable et al., 1991a).

Sodium (Na\(^+\)) is an extracellular ion that present in the most abundant quantity and plays an important role in controlling fluid and electrolyte balance. It exerts significant osmotic pressure; therefore, sodium is inseparably influenced both blood volume and blood pressure. So, it is capable to increase myocardial contractility and cardiac efficiency by elevating extracellular volume more than the transfused amount (Baue et al., 1967a; Velasco et al., 1980; Constable, 1999). Though no direct measurements of cardiac contractility were performed in the present study, an increase in heart rate and mean arterial pressure followed the infusion of HSS in the septicemic buffaloes seemed compatible with the concept of increased myocardial contractility. Hypertonic saline solution administration to septicemic buffaloes induced significant increase in serum sodium and chloride concentration which were accompanied by a transient decrease in serum potassium concentration. The increased concentration of serum sodium and chloride ions attributably increased serum osmolality which rapidly increased plasma volume by attracting fluid from intracellular space into interstitial and vascular spaces (Luypaert et al., 1986; Constable et al., 1991b). However, an increased concentration of sodium in HSS can cause salt poisoning (Tyler et al., 1994; Ajito et al., 1999). In this study, administration of 4 ml/kg of HSS produced transient high sodium level and animals were hypernatremic (164 ± 7.92 mmol/L) at t=3 h but its value decreased rapidly to become normal within t=24 h and no adverse effects were observed.
These results are inline with the results reported by other scientists (Constable et al., 1991b; Tyler et al., 1994; Constable et al., 1996; Suzuki et al., 1998). The mild decrease in potassium concentration after HSS infusion has been observed previously (Constable et al., 1991b; Suzuki et al., 1998), but was not considered clinically important. The buffaloes of the group B treated with HSS also felt thirsty until they were offered water.

Most of the knowledge about the effects of hypertonic saline originates from studies of haemorrhagic shock but septic shock differs from that condition in some aspects. Septic shock is more of a distributive shock with profound effects on peripheral resistance and with pronounced microcirculatory disturbances and vasodilatation due to endothelial and coagulation pathology, resulting in shunting and dysoxia in peripheral tissues. The goals in managing the haemodynamic changes in septic shock are to restore the intravascular volume, support cardiac performance and above all ensure oxygen delivery to peripheral tissues in balance with its demands. In this study, decreased pressure of arterial oxygen (PaO\textsubscript{2}) and increased pressure of venous carbon dioxide (PvCO\textsubscript{2}) were noted in buffaloes suffering from HS which clearly indicate hypoxic condition of the animals. Hypoxemia can be caused by hypoventilation or by diffusion impairment such as pulmonary oedema, shunting or ventilation-perfusion inequalities and it could also be due to myocardial pump failure resulting in decrease cardiac output. Hypertonic saline solution reduces pulmonary oedema (Constable et al., 1991b; Suzuki et al., 1998), increase myocardial contractility (Constable et al., 1991a; Tyler et al., 1994) and ultimately improve arterial oxygen pressure for its smooth delivery to the peripheral organs to avoid multiple organ dysfunction (MOD). During septic shock, polymorphonuclear cells activate and produce endothelial cell oedema in the endothelium of postcapillary venules. This leads to narrowing of capillary lumen and cause obstruction in the blood flow and reduction in oxygen transport (Corso et al., 1998; Oliveira et al., 2002). Hypertonic saline solution is also hypothesized to induce endothelial cell shrinkage, potentially increasing the inner capillary diameter and reducing hydraulic resistance, resulting in improved tissue perfusion (Suzuki et al., 1998; Oliveira et al., 2002). The relative increase in oxygen pressure following HSS infusion appeared greater than that observed without HSS in this study. Other investigators have demonstrated significant increases in oxygen pressure following administration of HSS to
endotoxic animals (Constable et al., 1991b; Suzuki et al., 1998; Ajito et al., 1999; Batmaz et al., 2003; Somell et al., 2007). The other cause of pulmonary responses may be linked to endotoxin-induced activation of host granulocytes and release of endogenous arachidonic acid metabolites, such as thromboxane and prostaglandin F$_{2\alpha}$, which are important contributors to endotoxin-induced pulmonary injury. Endotoxins may be linked to activation of host granulocytes and release of endogenous arachidonic acid metabolites, such as thromboxane and prostaglandin F$_{2\alpha}$, which are important contributors to hypovolaemia leading to endotoxaemia. Other than these, decreased venous blood pH and bicarbonates (HCO$_3^-$) during HS were indicators of acidosis in buffaloes. As flunixin meglumine is a potent inhibitor of arachidonic acid metabolites (Plumb, 1999), so, it helped to recover the animals from acidosis effectively.

Decreased serum protein, albumin and globulin were noted in septicaemic buffaloes. During endotoxaemia, hypoproteinemia might result from loss of protein-rich fluid into the extravascular space that develop simultaneously with intravascular haemolysis, or alternatively, from haemorrhage into tissues as a result of disseminated intravascular coagulation (Morris et al., 1986). The effect of endotoxins on vascular permeability is well recognized, and increased permeability probably results in loss of fluid accompanied by serum protein losses. Hypertonic saline administration exerts osmotic pressure and attracts fluid from extracellular compartment into the interstitial space resulting in increased concentration of serum protein, albumin and globulin which were also observed in the previous studies (Tyler et al., 1994; Constable et al., 1996).

The higher recovery rate in animals of group B was only due to the administration of hypertonic saline solution while buffaloes treated with the conventional treatment did not show satisfactory recovery rate because animals of that group were not administered with any fluid. Repetition of Ceftiofur HCl at 12 hour interval is two orders of the frequency recommended by manufacturer instead of 24 hours intervals because our own observations and those of others (Raza et al., 2000) would indicate that the HS cases respond temporarily with partaking of feed and water but worsens again if the repetition is deferred until 24 hours.
5.2. Evaluation of Hypertonic Saline Solution in the Treatment of Induced and Spontaneous Calf Scour

The incidence of mortality in the buffalo calves in Pakistan has been reported as high as 38.08% (Khan et al., 2009). The calf mortality is attributed to neonatal calf diarrhoea, pneumonia and pneumo-enteritis (Khan and Khan, 1996; Khan and Khan, 1992). Majority of the deaths are due to neonatal calf diarrhoea (Gitau et al., 1994). The role of enteropathogenic *E. coli* in neonatal buffalo calf diarrhoea is an establish factor, (Hafiz et al., 1994; Khan and Khan, 1996). Enteropathogenic *E. coli* adheres to the mucosa, proliferates in intestinal lumen and produces a potent enterotoxin stimulating excessive fluid secretion (Khan and Khan, 1995). This loss of fluid from intestinal mucosa causes the principle sign (diarrhoea) which often leads to dehydration. As a result of dehydration, acid-base imbalance, excessive loss of water and electrolytes and hypovolaemia takes place consequently resulting onto shock and death (Roussel and Kasari, 1990). According to Hudson and Johnson (1986), as much as 15% loss of fluid results in death.

In the present study, comparative efficacy of two crystalloids (isotonic and hypertonic saline solutions) in combination with an antibiotic (ceftiofur HCl) and a NSAID (flunixin meglumine) was evaluated in neonatal diarrhoea in buffalo calves induced by the oral administration of broth culture of *E. coli*. The same treatment protocols were then trialed on the spontaneous occurring neonatal buffalo calf diarrhoea. The protocol to induce neonatal diarrhoea promoted severe diarrhoea in 100% of the calves. A moderate dehydration around 8%, hyponatraemia, hypochloraemia, hypokalaemia, decreased arterial oxygen pressure, blood pH, bicarbonates and increased body temperature, heart rate, partial pressure of carbon dioxide were recorded during induced neonatal diarrhoea. The calves with spontaneous neonatal diarrhoea exhibited 10% dehydration of and higher severity of other parameters mentioned above. However, respiration rate and serum potassium ions concentration showed reverse trend than induced calf scour.

Dehydration in calves with neonatal diarrhoea is accompanied by a decrease in extracellular fluid volume with resulting decrease plasma volume (Walker et al., 1998).
The aim for the treatment of severely dehydrated calves with neonatal diarrhoea is to restore plasma volume rapidly, thereby improving cardiac output, increasing mean arterial pressure and oxygen delivery, and correcting acid-base and electrolyte imbalance. Plasma volume expansion could be achieved by administering the fluid through different routes including oral, subcutaneous, intraperitoneal or intravenous route or a combination thereof. Oral administration of electrolyte solutions could be successful in the resuscitation of moderately dehydrated calves, but severely dehydrated calves require more rapid replacement of sodium and free water deficits. Orally administration of electrolyte solutions resuscitates the animals slower than that achieved through IV fluid administration, because a limited fluid volume could be accommodated by gastrointestinal tract. Resuscitation accomplished by subcutaneous administered fluids is slower because 6-8 hour period is required for absorption in normal animals but it is greatly delayed in diarrhoeic animals because of marked decrease in cardiac output and peripheral blood flow. Intraperitoneal administration of fluids is not recommended for resuscitating severely dehydrated calves because of the risk of peritoneal adhesions. Contrarily, the intravenous route is an effective and safe to deliver large quantity of fluids needed to rapidly resuscitate severely dehydrated diarrhoeic calves.

Infusion of isotonic saline solutions (ISS) is widely accepted as a gentle approach to treat severely dehydrated calves with diarrhoea effectively. It is recommended @ 90 mL/kg BW which is a large volume to infuse throughout a 3 to 4-hour period to correct hydration status. Intravenous administration of ISS requires periodic monitoring of the flow rate. It is often difficult to maintain a constant flow rate because of fluctuation between standing and recumbent calves and stretching and tangling of fluid line that result from activity and movement of calves. Catheter patency is another factor that is difficult to maintain. Although catheter-related problems can be timely identified and corrected in a hospital setting because of frequently monitoring, but these are more likely to go undetected for long periods in on-farm situations, which cause delay in the fluid administration needed by dehydrated calves. So, a more practical method for IV administration of fluids would be advantageous. Rehydration with hypertonic saline (7-7.5%) solution represents an alternative for resuscitation of diarrhoeic calves when an infusion treatment cannot be carried out for any reason. The primary therapeutic
approach is based on a replenishment of the intravascular volume from the intracellular compartments caused by an increased plasma osmolarity, thereby improving cardiac output and increasing systemic oxygen delivery.

The protocol to induce neonatal diarrhoea caused mortality in calves and hypertonic saline treated calves of group B showed increased survival rate than group A. The percent survival among animals treated with protocol A and B was 75 and 83.3, respectively. Difference in recovery rate between these protocols was non-significant (Table 6). However, spontaneous cases of neonatal diarrhoea showed increased mortality in group A when compared to group B which showed significant difference over group A). The percent survival among animals treated with protocol A and B was 58.3 and 75, respectively. Difference in recovery rate between these protocols was significant (Table 8). The higher recovery rate in group B than group A was attributed to the use of hypertonic saline solution in the former treatment protocol.

Hypertonic saline solution permits rapid expansion of plasma volume and correction of sodium deficit than that achieved with administration of a high volume of ISS, even when administered at an aggressive dose rate (90 mL/kg/h) is used (Constable et al., 1996; Senturk, 2003). As early mentioned that fluid loss in diarrhoeic calves is principally extracellular and sodium is the predominant cation in extracellular fluid, so, resuscitation requires replacement of sodium deficit as quickly as physiologically possible which is provided by HSS.

In our study, increased heart rate was observed in induced and spontaneous diarrhoeic dehydrated 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 (Constable et al., 1995; Flores et al., 2006). In calves with induced diarrhoea, heart rate decreased toward baseline after administration of isotonic and hypertonic saline solutions to groups A and B, respectively. In spontaneous calf scour, the group treated with HSS showed elevated heart rate for about 1 h compared to basal values. 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; Cambier et al., 1997). These effects were, however, transient; lasting only for one hour and then heart rate dropped and became normal rapidly.

Our results have shown increased haematocrit and Hb concentration in dehydrated diarrhoeic calves. These results had also been reported by the previous studies as increase in Hct and Hb concentration is the cause of decreased plasma volume (Constable et al., 1991b; Tyler et al., 1994; Senturk et al., 2003). In the studies by Kristensen et al. (1990) and Constable et al. (1991b), where intervention with a fixed volume of HSS with or without dextran was administered after endotoxin infusion, an immediate response to fluid treatment with a decrease in haematocrit and Hb concentration were found indicating expansion in intravascular volume. In our study, we also found a decrease of Hct and Hb concentration in both groups, indicating that the fluid resuscitation with HSS was effective for plasma volume expansion.

Hypertonic saline solution caused immediate and significant increase in mean arterial and central venous pressures which were decreased in calves with neonatal diarrhoea. This is most likely a volume resuscitation effect associated with increased preload and reported inherent positive inotropic effect of hypertonic saline solutions (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, 1998). Some studies (Constable et al., 1996; Rocha e Silva, 1998; Walker et al., 1998) recommended the use of 6% dextran-70 in association with hypertonic saline solution to maintain plasma expansion. However, our results corroborate the viability of HSS without dextran. These findings of our study are also supported by the results reported by Flores et al. (2006).

Blood gas analysis is 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 this study, decreased arterial oxygen pressure (PaO₂), increased venous
carbon dioxide pressure (PvCO₂), decreased blood pH and bicarbonates were noted in calves suffering from neonatal diarrhoea either it was induced or natural. Decreased PaO₂ indicated that animals were suffering from hypoxia while other results were in favour of calves with acidemia. Hypoxemia could be due to the endothelial cell oedema in the endothelium of postcapillary venules which cause obstruction in the blood flow and reduction in oxygen transport (Corso et al., 1998; Oliveira et al., 2002). Hypertonic saline solution is also hypothesized to induce endothelial cell shrinkage resulting in improved tissue perfusion and oxygen delivery (Suzuki et al., 1998; Oliveira et al., 2002). Hypertonic saline infusion to the calves of group B also decreased the pressure of CO2 resulting in blood pH and bicarbonates increase which helped in recovering the calves from acidosis. These results were supported by the previous studies (Constable et al., 1991b; Senturk, 2003; Somell et al., 2007).

Hyponatraemia was observed in diarrhoeic calves also reported in several studies (Constable et al., 1996; Berchtold 1999; Naylor 1999; Flores et al., 2006). In contrast with the treatment with normal saline solution, an immediate increase in serum sodium concentration was observed in calves treated with HSS. In animals with normal natraemia, no evidence of severe hypernatraemia has been reported after using a dose of 4 mL/Kg BW, at the infusion rate employed (Cambier et al., 1997; Rocha e Silva, 1998; Ajito et al., 1999; Koch and Kaske, 2008). In our study, the highest serum sodium after the initial fluid administration was 143.5 ± 10.75 mmol/L. However, hypertonic saline solution is contradicted in chronic symptomatic or asymptomatic hyponatraemia as it causes neurological complications (Dibartola, 2000). In our study, no abnormality in the behavior and attitude after the treatment in calves submitted to the infusion of hypertonic saline solution.

5.3. Evaluation of Hypertonic Saline Solution in the Treatment of Dehydrated Diarrhoeic Goats and Buffaloes

The potential benefits of hypertonic saline solution (7.5% NaCl; 4 mL/kg BW) were evaluated in the resuscitation of dehydrated diarrhoeic goats (n=24) and buffaloes (n=24). The study was conducted on clinical cases of diarrhoea. The most conspicuous
findings of this study related to the substantial improvement in partial pressure of arterial oxygen, haemodynamic improvements and reversing acidemia.

Hypertonic saline solution was safely administered to the goats and buffaloes with diarrhea. No adverse effects were observed during the study period. Diarrhea in goats and buffaloes is one of the most common disease conditions which significantly cause economy losses. Treatment of diarrheic goats and buffaloes is always a complex problem. Dehydration and electrolyte losses and imbalance in animals with diarrhea are common causes of mortality. Successful treatment of diarrheic animals depends primarily on the administration of fluids and electrolytes (Radostits et al., 2007). For this purpose, various treatment regimens have been tried on animals suffering from diarrhea and a multitude of different drugs are still used. In the present study, hypertonic saline solution was evaluated along with ceftiofur and flunixin and compared its effects with large volume of isotonic saline solution.

In the present study, mortality was recorded in diarrheic goats but no mortality was observed in buffaloes. Less mortality was recorded in hypertonic saline treated goats of group B and it increased survival rate when compared to group A. The percent survival among goats treated with protocol A and B were 50 and 75, respectively. Difference in recovery rate between these protocols was significant (Table 10). The higher recovery rate in group B than group A was attributed to the use of hypertonic saline solution in the former treatment protocol. However, no mortality was observed in diarrheic buffaloes during the study period.

Diarrhoea caused hypotension in the goats which was characterized by decreased MAP and CVP. Decreased MAP and CVP may be interpreted as an indicator of hypotension, decreased plasma volume and hypovolaemia. Cohen et al. (1996) reported that typical hypotension during hypovolaemia and/or endotoxaemia is due to decrease in plasma volume. Administration of HSS causes expansion in the plasma volume which ultimately increases MAP. The increased arterial blood pressure helps to improve cardiac output which increases central venous pressure (Constable et al., 1996; Walker et al., 1998). In our study, MAP and CVP increased after infusion of HSS more rapidly than
isotonic treated group. These findings are in line with the findings of previous studies (Constable et al., 1996; Walker et al., 1998; Somell et al., 2007).

Blood gas analysis is 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. Metabolic acidosis is the result of an accumulation of metabolic waste products and lack of buffer system in hypovolaemia. In the present study, decreased pressure of oxygen was observed accompanied by increase in carbon dioxide (PaCO$_2$). Other than these, decreased venous blood pH and decreased bicarbonates (HCO$_3^-$) during diarrhoea were indicators of metabolic acidosis in goats and buffaloes. Administration of ISS and HSS recovered the animal from acidosis but better results were observed in HSS treated groups in goats and buffaloes. Previous studies also reported the affectivity of HSS in reversing the acidemia (Ajito et al., 1999; Constable, 1999; Kreimeier et al., 1991).

Hypertonic saline solution infusion induced significant increase in serum sodium and chloride concentration, which were accompanied by a transient decrease in serum potassium concentration. The key feature for successful resuscitation of diarrhoeic animals is the total amount of sodium (Constable, 1999). In group B of diarrhoeic buffaloes, the sodium ions concentration increased beyond the limit of hypernatraemia that is 160 mmol/L (Tyler et al., 1994), but this increase was temporary and then these values became below this level within 6 hours. So, infusion of HSS cause hypernatraemia but not for a prolonged period suggesting that it could be safely use in buffaloes with diarrhoea (Constable et al., 1991; Tyler et al., 1994; Ajito et al., 1999). On the other hand, sodium concentration did not increased cut-off point of hypernatraemia after administration of HSS to hyponatraemic goats due to diarrhoea. The mild decrease in potassium concentration after HSS infusion has been observed previously (Constable et al., 1991; Suzuki et al., 1998), but was not considered clinically important.

The findings of this study suggest that hypertonic saline solution in combination with antibiotic (ceftiofur HCl) and NSAID (flunixin meglumine) is a rapid and effective method resuscitation of severely dehydrated goats and buffaloes. It is similar in effectiveness to conventional treatment in which isotonic saline solution along with
antibiotic and NSAID are used for their resuscitation. However, further evaluations are required to appraise the real impact of this alternative resuscitation strategy in diarrhoea.

Conclusions

On the basis of findings of this study, it is tempting to suggest that hypertonic saline (7.5% NaCl @ 4 mL/kg BW) solution can be safely administered to buffaloes suffering from haemorrhagic septicaemia, buffalo calves dehydrated due to neonatal diarrhoea, dehydrated diarrhoeic goats and buffaloes.

It offsets deleterious haemodynamic effects of endotoxins, thus ameliorates the septic shock more effectively than does antibiotic+NSAID therapy alone in HS.

In addition to being rapid and effective, intravenous administration of a small volume of HSS provides a practical and economical method to resuscitate calves dehydrated due to neonatal diarrhoea, diarrhoeic goats and buffaloes.

In view of preliminary nature of the present study (with a particular reference to the use of HSS in HS therapy), additional work on use of HSS in disease conditions associated with hypovolaemia and/or endotoxaemia is clearly warranted.
There are a number of diseases of livestock especially buffalo which adversely affect its health and one associated with considerable pecuniary losses due to mortality. Of these, haemorrhagic septicaemia (HS) and calf scour are the major diseases causing huge economic losses to the country each year. Haemorrhagic septicaemia is ranked as the number one killer of buffaloes in Pakistan. Although routine vaccination against HS is practiced in the field, many animals remain unvaccinated and they are likely to contract the disease. This disease is associated with gram negative bacterium which releases endotoxins in the blood. These endotoxins are the major contributors in the development of this disease which affect central circulatory system profoundly through decreasing mean arterial blood pressure and cardiac output. In the field, treatment of HS fails partly because affected animals are not given fluid therapy as a single animal requires a large volume of isotonic fluids (about 40-50 litres). To this end, there is a need for an alternative fluid that should be a small-volume resuscitative and should be easily applicable in the field. Hypertonic saline (7-7.5% NaCl) solution (HSS) has been used successfully as a small-volume resuscitative in sheep, horses, dogs, cats, humans and calves with hypovolaemic and endotoxic shock. This study was planned to evaluate the efficacy of HSS as an adjunct to antibiotic therapy and NSAID in the treatment of clinical cases of haemorrhagic septicaemia in buffaloes (*Bubalus bubalis*), induced scour in buffalo calves and other clinical conditions (spontaneous scour in buffalo calves, diarrhoeic dehydrated goats and buffaloes) of livestock characterized by hypovolaemia and/or endotoxaemia including spontaneous cases of scour in buffalo calves, diarrhoeic dehydrated goats and buffaloes.

In the first part of the study, efficacy of HSS was evaluated in the field cases of HS in buffaloes. For this purpose, 50 buffaloes suffering from HS were selected from the field. The disease was diagnosed on the basis of clinical signs. Then these animals were randomly divided into two equal groups (A and B). Buffaloes of group A were treated
with the conventional HS treatment already in vogue i.e. ceftiofur HCl and flunixin meglumine @ 6 mg/kg and 2 mg/kg BW, IM and IV, respectively. Group B was treated with intravenous infusion of hypertonic saline solution @ 4 mL/kg BW followed by isotonic saline solution @ 10 mL/kg BW along with ceftiofur HCl and flunixin meglumine. The efficacy of treatment was evaluated on the basis of survival rate, severity index of the disease, clinical parameters, haematological analysis, haemodynamic parameters, blood gas analysis, serum electrolytes and serum biochemical profiles. These all evaluation parameters were recorded at baseline (during disease), t=1, t=3, t=6, t=12, t=24 and t=36 hours after treatment. Survival rate in group B (80%) was significantly higher ($P < 0.05$) than that in group A (52%). Disease severity index also decreased significantly in hypertonic saline treated buffaloes (group B) at each observational hour and showed significant difference ($P < 0.05$) over group A throughout the study period. High rise in body temperature was noted in all the diseased buffaloes which decreased significantly in both the groups, albeit no significant difference was observed between two groups except at t=24 h whereat group B showed statistical difference over group A. Respiration increased after treatment in both groups but group B showed better recovery toward normal and showed significant difference ($P < 0.05$) over group A. Heart rate showed similar trend as that of respiration rate in returning to normal values and group B showed significant difference ($P < 0.05$) over group A. Values of haematocrit and haemoglobin concentration were better recovered by group B and showed significant differences ($P < 0.05$) over its counterpart. Hypertonic saline administration (group B) significantly increased mean arterial (MAP) and central venous pressures (CVP) as compared to conventional treatment (group A). Mean arterial pressure was restored at t=3 h after infusion of HSS in buffaloes of group B, while conventional treatment protocol failed to restore it even at t=36 h. Similarly, CVP was also restored at t=12 h in group B, while group A failed to restore it throughout the study period. Partial pressures of arterial oxygen and venous carbon dioxide were better recovered in group B and significantly differed ($P < 0.05$) in their values in group A, albeit group A also registered a return in these parameters near to the reference values within the experimental period. Blood pH and bicarbonates decreased after HSS infusion in group B followed by a rapid increase towards normal and became normal within experimental period. Contrarily, in group A
blood pH and bicarbonates failed to attain their normal values. Sodium ions concentration rose to the hypernatraemic cut-off point (160 mmol/L) after HSS infusion in group B, but it was transient and decreased after t=3 h and became normal within experimental period. Similar trend was observed in chloride ions concentration, while potassium ions concentration recovered better in group A at the start but at t=36 h, group B showed better recovery to attain the normalcy in the serum concentration of potassium ions. Serum biochemical profiles (total protein, albumin and globulin) increased significantly (P < 0.05) after infusion of HSS (group B) and differed significantly (P < 0.05) from those of group A.

Scour in neonatal calves is another serious welfare problem and considered as an important cause of economic losses. It remains an important and major cause of illness and death in buffalo calves in Pakistan. The efficacy of HSS was also evaluated in the resuscitation of induced as well as in spontaneous cases of scour (neonatal calf diarrhoea) in buffalo calves (n=24 each). The neonatal diarrhoea was induced through oral administration of 2 mL broth culture of enteropathogenic E. coli (coliform count of 10^{10} CFU/mL). The evaluation criteria and recording intervals were same as those in HS. Buffalo calves suffering from induced and spontaneous calf scour were randomly divided into two equal groups viz. A and B. The treatment protocols for both induced and spontaneous cases of calf scour were i.e. isotonic saline (ISS; group A) infusion @ 90 ml/kg BW and HSS infusion @ 4 mL/kg BW (group B) along with ceftiofur HCl @ 6 mg/kg BW and flunixin meglumine @ 2 mg/kg BW. After treatment, both the groups showed similar trend in recovering the body temperature, respiration rate and heart rate; the differences between two groups being statistically non-significant. Similar case was also observed in haematological parameters after instituting the treatment and the increased values of haematocrit and Hb concentration during diarrhoea returned to their normal within the experimental period. In both categories of calf scour, HSS infusion to the calves (group B) restored mean arterial pressure and central venous pressure significantly (P < 0.05) earlier than the ISS infusion (group A). Hypertonic saline administration to (group B) also showed better results in restoration of partial pressures of arterial oxygen and venous carbon dioxide and differed significantly (P < 0.05) from group A. The values of blood pH and bicarbonates differed non-significantly between the
two groups. Hypertonic saline infusion affected a significant increase in serum sodium and chloride concentrations at t=3 h, while group A showed a slower trend towards the recovery. However, the values became normal within the experimental period. Induced calf scour was associated with hypokalaemia which was tempered by both treatment protocols; the difference between the two treatments being statistically non-significant. While hyperkalaemia was noted in spontaneous cases of calf scour and group B showed significant difference (P < 0.05) over group A in recovering the serum concentration of potassium ions. Serum biochemical profiles recovered in both treatment groups without any statistically valid difference.

The resuscitation of diarrhoeic dehydrated goats (n=24) and buffaloes (n=24) through administering HSS and ISS was evaluated as one of the parts of the study. The clinical cases of diarrhoeic goats and buffaloes were included in this study and were divided randomly into two equal groups i.e. A and B. The treatment protocols were same as that in spontaneous cases of scour in buffalo calves. Both treatment protocols helped in recovering the normal values of all the parameters studied within experimental period. Nonetheless, HSS treated animals (group B) showed significant differences (P < 0.05) over group A in recovering the values of heart rate, MAP, CVP, partial pressure of arterial oxygen, blood pH and bicarbonates in diarrhoeic goats. In diarrhoeic buffaloes, HSS infusion only showed significant difference (P < 0.05) in recovering partial pressure of venous oxygen to group A, while other parameters were recovered to normal without any statistical difference.

In conclusion, on the basis of findings of this study, it is tempting to suggest that hypertonic saline (7.5% NaCl @ 4 mL/kg BW) solution can be safely administered to buffaloes suffering from haemorrhagic septicaemia, buffalo calves dehydrated due to neonatal diarrhoea, dehydrated diarrhoeic goats and buffaloes. It offsets deleterious haemodynamic effects of endotoxins, thus ameliorates the septic shock more effectively than does antibiotic+NSAID therapy alone in HS. In addition to being rapid and effective, intravenous administration of a small volume of HSS provides a practical and economical method to resuscitate calves dehydrated due to neonatal diarrhoea, diarrhoeic goats and buffaloes. In view of preliminary nature of the present study (with a particular
reference to the use of HSS in HS therapy), additional work on use of HSS in disease conditions associated with hypovolaemia and/or endotoxaemia is clearly warranted.
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