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Effects of hypertonic solutions on biochemical parameters and endotoxemic calves resuscitation

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Abstract

Endotoxemia is a common and life threatening inflammatory condition in calves which can lead to shock, multiple organ dysfunction, and immunosuppression and biochemical dyshomeostasis. In order to correct peripheral perfusion and metabolic disturbances, hypertonic fluids are recommended as part of treatment. The aim of the present study was to determine the resuscitating effects of hypertonic solutions (NaCl 7.2% and NaHCo3 8.4%) on endotoxemia-induced biochemical alterations. Nine Holstein male calves were randomly divided into three groups (control; n=3 and two treatment; n=6). All calves were injected bacterial Lipopolysaccharide (LPS) and were monitored for four hours post administration (p.a) at several regular intervals. Biochemmical parameters such as Total protein (TP), Fibrinogen (Fib), Lactate, Cortisol and Adrenocorticotropic hormone (ACTH) were measured as well as Rectal Temperature (RT), Respiratory Rate (RR) and Heart Rate (HR). Approximately, blood biochemistry showed significant change in all parameters following endotoxemia which were corrected by hypertonic solution interventions. The present study demonstrated that hypertonic sodium bicarbonate exerted its resuscitating effects by more abrupt increase of ACTH and Cortisol levels.

Key words: Endotoxemia, Resuscitation, Calve, Hypertonic solution

Introduction

Endotoxin is a major compartment of the outer membrane of Gram-negative bacteria and endotoxemia is a potentially devastating complication of several diseases of calves e.g. enteric disease, septicemia and pneumonia (Beutler *et al.* 2003). Endotoxemia is a serious inflammatory condition which can lead to shock, multiple organ failure, immunosuppression, etc (Schroeder *et al.* 2005). Being absorbed to the circulation, endotoxin causes various adverse effects including cardiovascular compromise, lactic acidiosis, leukopenia, glucose dyshomeostasis, hemostatic alteration, gastrointestinal, respiratory and renal disturbances (Schumann *et al.* 1990).

Biochemically, endotoxicosis initially includes endocrine-metabolic stress response such as hyperlactemia, hyperglycemia and plasma rise in cortisol level, followed by a dose-dependent response including hypoglycemia, leukopenia and thrombocytopenia (Cullor 1992; Griel *et al.* 1975; Luthman *et al.*1988). Such reaction is generally known as the acute-phase response due to the rapid activation of the inflammatory process.

The traditional treatment for endotoxemic animals is correction of cardiac and pulmonary dysfunction, elimination of causative bacteria and reduction of endogenous mediators (Singh et al. 2002). Fluid therapy, in endotoxemic shock, causes rapid intravascular expansion volume, rising mean arterial pressure (MAP), cardiac output and perfusion. In septic shock caused by bacterial wall LPS, due to endothelial damages, blood vessels become leaky, allowing fluid to seep out into the tissues, resulting in tissue edema. Hypertonic solutions by increasing plasma osmolarity shifting water from interstitial spaces into the intravascular spaces (Zornow, 1996). Initially, in 1938, hypertonic solutions were applied in human (Kline *et al.* 2005), and in 1980 small volume of saline hypertonic solutions was successfully used for haemorrhagic shocks (Zornow, 1996). The hypertonic solutions are positive inotrope, which increases the osmolarity and corrects base-excess. The sodium chloride hypertonic solution was demonstrated as a resuscitating practice in calves (Annane *et al*. 2007; Mauricto *et al.* 1997) that causes 2-4-fold volume expansion (Helwig *et al.* 1938; Semrad *et al.* 1993).

Administration of hypertonic sodium bicarbonate instead of isotonic solutions causes more rapid pH increase, hypokalemia and hypernatremia, since it does not require intracellular metabolism for alkalinizing effect (Constable *et al*. 1999; Tollofsrud *et al*. 2001; Zhang *et al.* 2008). In addition, hypertonic solutions stimulate hypothalmus-hypophysis axis which induce ACTH and cortisol and Antidiuretic hormone release, giving rise to increasing blood pressure (Annane *et al.* 2005; Prigent *et al*. 2004). The aim of the current study was to investigate the biochemical alterations following administration of hypertonic solutions, and determining the role of ACTH and cortisol hormones in resuscitating endotoxemic calves.

Materials and methods

Animals and experimental design

In this experiment, nine Holstein male calves were randomly divided into three groups (average age 75 ± 4 days): a control group (n = 3) and two treatment groups (each group 3 calves). The day before the experiment, the calves were weighed (84±5.0 kg), after which a 14 G indwelling catheter was placed aseptically in the left jugular vein. The clinical condition at 0 h was evaluated and a RT $\geq$ 40°C was handled as an exclusion criterion at this time. Individual hutches were prepared for all calves (1 m × 1.5× 1m) with free access to water.

All animals were kept in the same room with mean temperature of 18-20 °C and 12 hour/day light (07:00 to 19:00 h). Normal feeding management included 2.5kg concentrate (40% barley, 20% corn, 18% soybean, 19% bran, 1.5% mineral and 1.5% vitamin supplements) twice a day (at 0800 and 1500 h). Fresh hay was offered shortly after concentrate and was freely accessible to all animals.

All calves were injected bacterial LPS (Escherichia coli O111:B4, L3012; Sigma, 0.5 μg/kg, IV) in 50-mL volume of Sodium chloride 0.9%. Most of the blood samples, for analysis of biochemical parameters, were collected in an EDTA tubes at just before (-1) and 5, 10, 15, 30, 60 and 120 minutes post administration (p.a) of endotoxin. At 120 minute, the isotonic NaCl 0.9%, hypertonic NaCl 7.2% and hypertonic $NaHCo_3$ 8.4% were rapidly injected to control, treatment 1 and treatment 2 groups respectively. Subsequently, following administration of the solutions, the blood samples were again collected at 125, 135, 150, 180 and 240 minutes. At all above mentioned sampling stages, rectal temperature (RT), respiratory rate (RR), and heart rate (HR) were recorded as well.

Serum biochemical determination

Blood samples collected in EDTA were centrifuged and the serum harvested. Total protein (TP) was measured by Automatic chemistry analyzer (*ELITech®).* For lactate levels measurement, blood samples were collected in a Perchloric acid-contained tube and centrifuged (10 min, 1500 g) and after centrifugation, the above liquid was extracted for spectrophotometry. Fibrinogen (Fib) was measured spectrophotometrically, in which the difference between clotted and normal plasma showed the exact fibrinogen value. Adrenocorticotropic hormone (ACTH) and cortisol levels were determined using

commercially available Elisa kits (*EASTBIOPHARM* ®).

Statistical analysis

The repeated measures ANOVA method was used to analyze the variables in each group during times; whereas, the one-way ANOVA method was applied to compare the three groups.

Results

In the present study, various biochemical parameters were evaluated in endotoxemic calves up to 4 hours post administration of bacterial Lipopolysaccharide. At 120 min of time, the specific solutions were injected to each group and the alterations were recorded. Administration of LPS to calves resulted in clinically relevant signs characteristic of transient systemic inflammatory response. Fig.1 indicates RT changes, rising at 15 min of LPS p.a and returning to normal after hypertonic solution injections, showing considerable drop at 150 min in treatment groups ($P < 0.01$); so, no significant difference was observed between treatment groups. The control group slowly decreased and did not reach the normal temperature at 240 min ($P<0.05$). The RR increased at time 0 and the maximum level was recorded in control group at 60 min (70 ± 5) which significantly remained higher than the treatment groups ($P<0.05$). The NaCl 7.2% group showed remarkable decline upon fluid therapy and reached 33±5. The HR indicated slight alterations in all groups with the peak in control group (125±5) at 125 min, but no statistically significant changes were recorded ($P<0.05$).

Lactate concentration gently increased at 15 min of endotoxin (p.a) and reached the peak at 120 min (42 mg/dl) in all groups, and dramatically decreased after treatments. In comparison with control and NaCl 7.2% groups, the significant results were obtained in NaHCo3 8.4% group at 240 min of time (19 mg/dl). Total protein declined slowly and reached 5.8 g/dl at 120min in all groups. The concentration returned to normal after treatments. Significant results were found in each group during the study; whereas, no significant changes were observed among groups. Fibrinogen concentration rose nearly 5 minutes post administration of endotoxin, reaching the peak at 120 min in all groups with the highest level in Sodium bicarbonate group (0.9 mg/dl). Conspicuous drop was observed after treatments, especially in $NaHCo_3$ 8.4% group at 150 min. Cortisol level started to increase at 30 min and reach

41µg/dl at 125 min in Sodium bicarbonate group and abruptly increased from 135 min and reached 46 µg/dl at 240 min (P < 0.05). In NaCl 7.2% group the increase trend continued up to 240 min (46 µg/dl) of time (P < 0.05). The ACTH level dramatically increased from 15min and reached 35 ng/ml at 120 min in Sodium bicarnonate group, reaching the maximum level up to 240 min (37 µg/dl)(P < 0.05). The NaCl 7.2% group showed lesser concentration of ACTH and cortisol (P < 0.05).

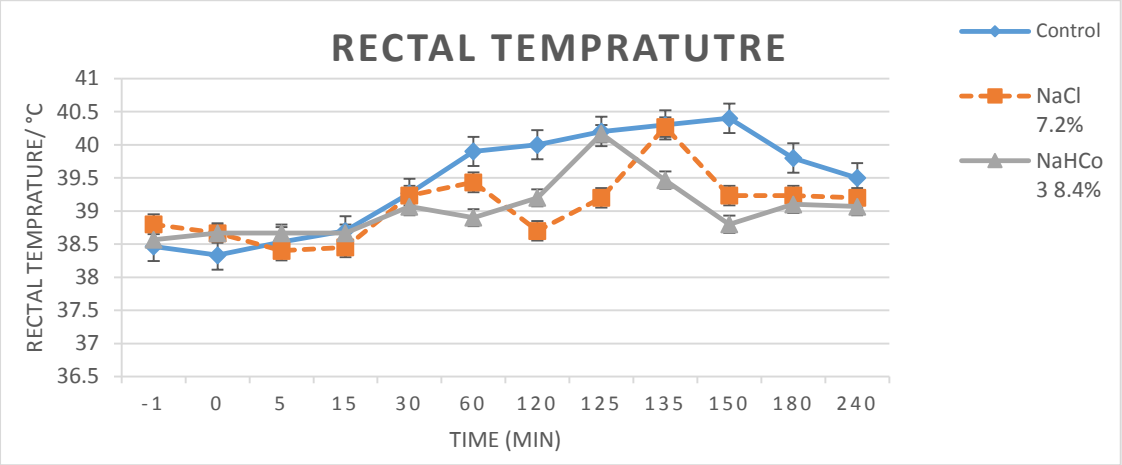

Fig 1: Mean (±SE) rectal temperature of calves at each sampling time before and after injection of lipopolysaccharide (LPS) in control (NaCl 0.9%) and treatment (NaCl 7.2% and NaHCo3 8.4%) groups. Significant differences were found (P < 0.05).

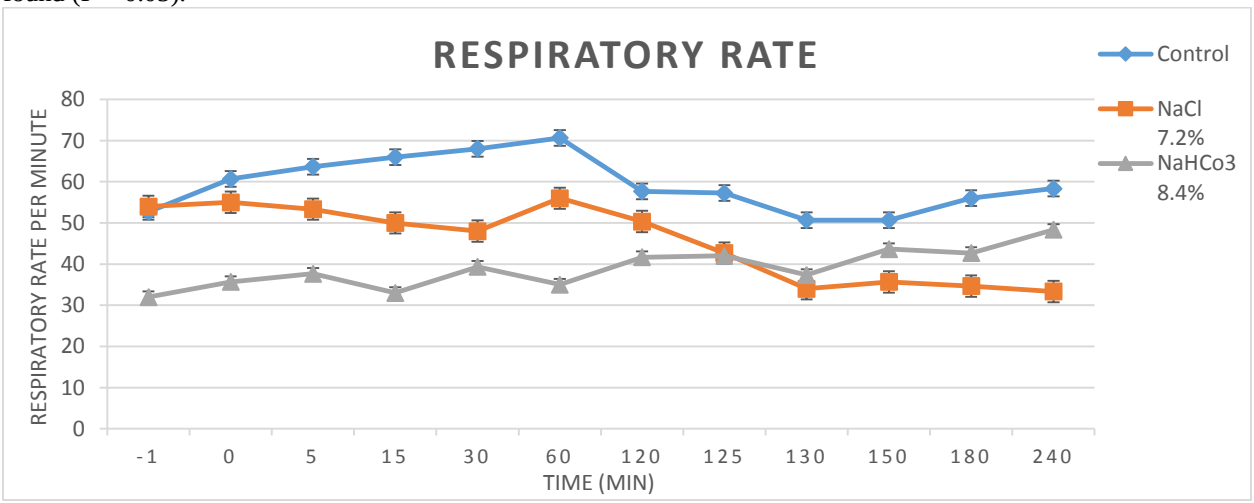

Fig 2: Mean (±SE) respiratory rate of calves at each sampling time before and after injection of lipopolysaccharide (LPS) in control (NaCl 0.9%) and treatment (NaCl 7.2% and NaHCo3 8.4%) groups. Significant differences were found (P < 0.05).

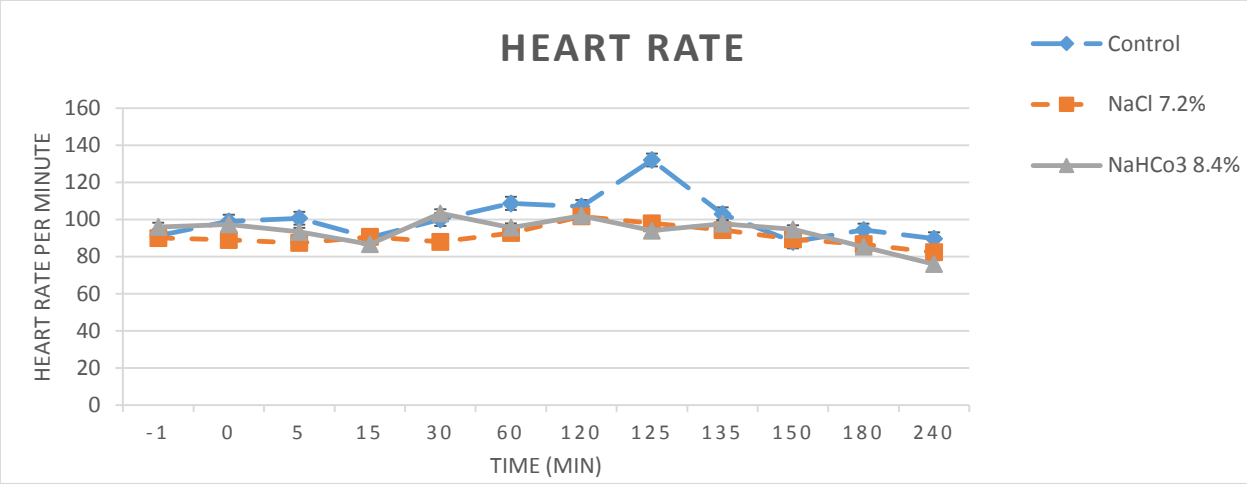

Fig 3: Mean (±SE) heart rate of calves at each sampling time before and after injection of lipopolysaccharide (LPS) in control (NaCl 0.9%) and treatment (NaCl 7.2% and NaHCo3 8.4%) groups.Significant differences were found (P > 0.05).

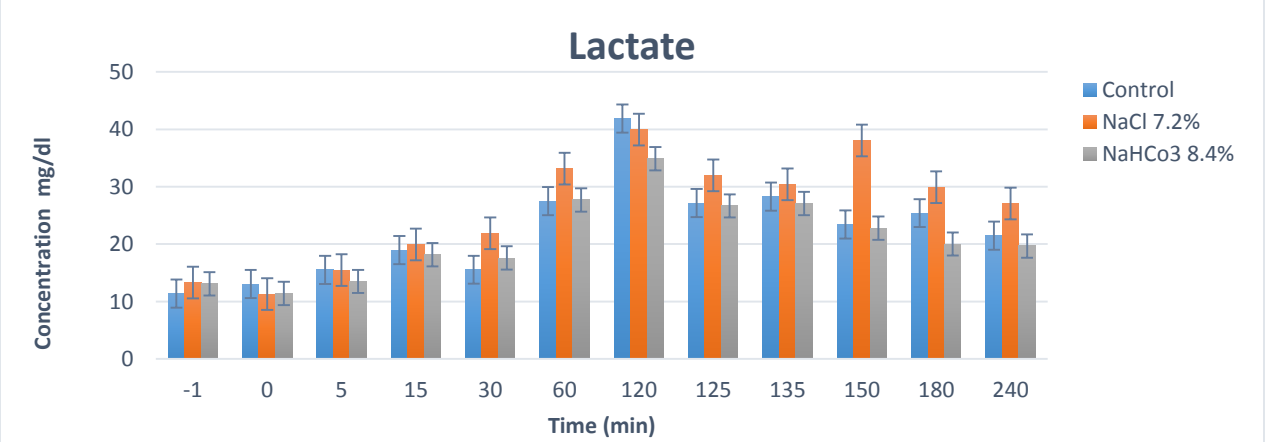

Fig 4: Mean (±SE) serum lactate concentration of calves at each sampling time before and after injection of lipopolysaccharide (LPS) in control (NaCl 0.9%) and treatment (NaCl 7.2% and NaHCo3 8.4%) groups. Significant differences were found (P<0.05).

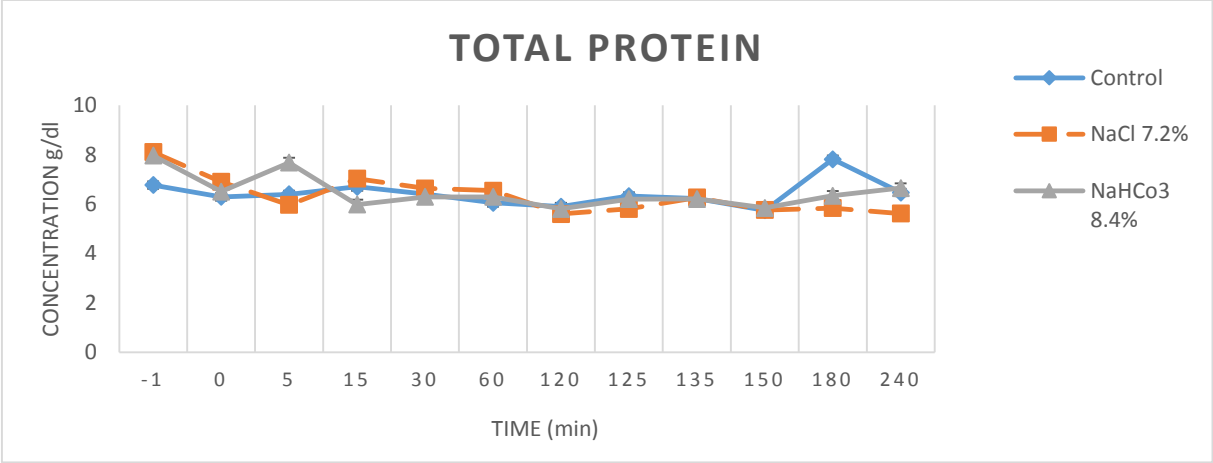

Fig 5: Mean (±SE) serum total protein concentration of calves at each sampling time before and after injection of lipopolysaccharide (LPS) in control (NaCl 0.9%) and treatment (NaCl 7.2% and NaHCo3 8.4%) groups. Significant differences were found (P < 0.05).

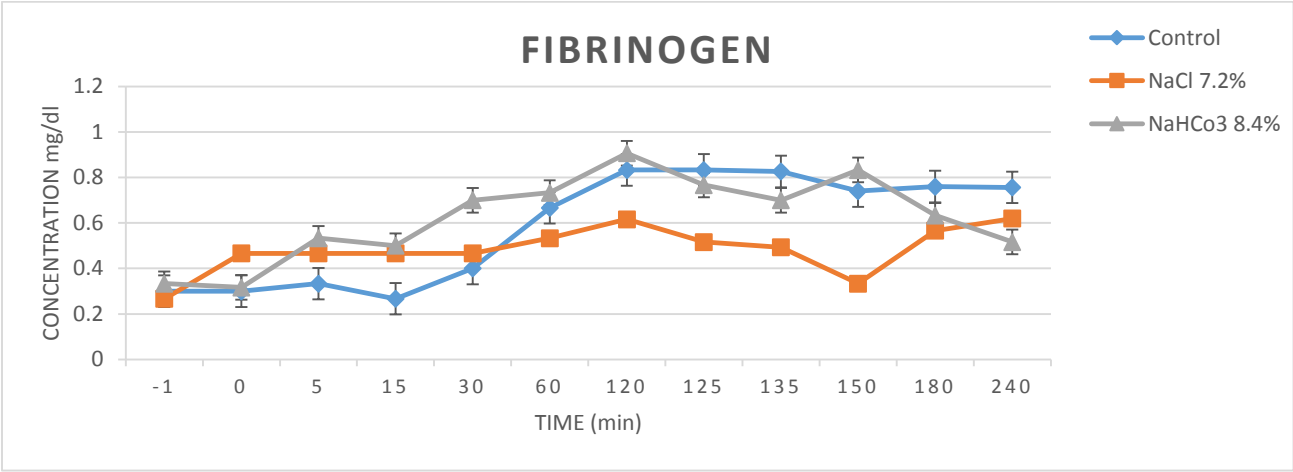

Fig 6: Mean (±SE) serum fibrinogen concentration of calves at each sampling time before and after injection of lipopolysaccharide (LPS) in control (NaCl 0.9%) and treatment (NaCl 7.2% and NaHCo3 8.4%) groups. Significant differences were found (P < 0.05).

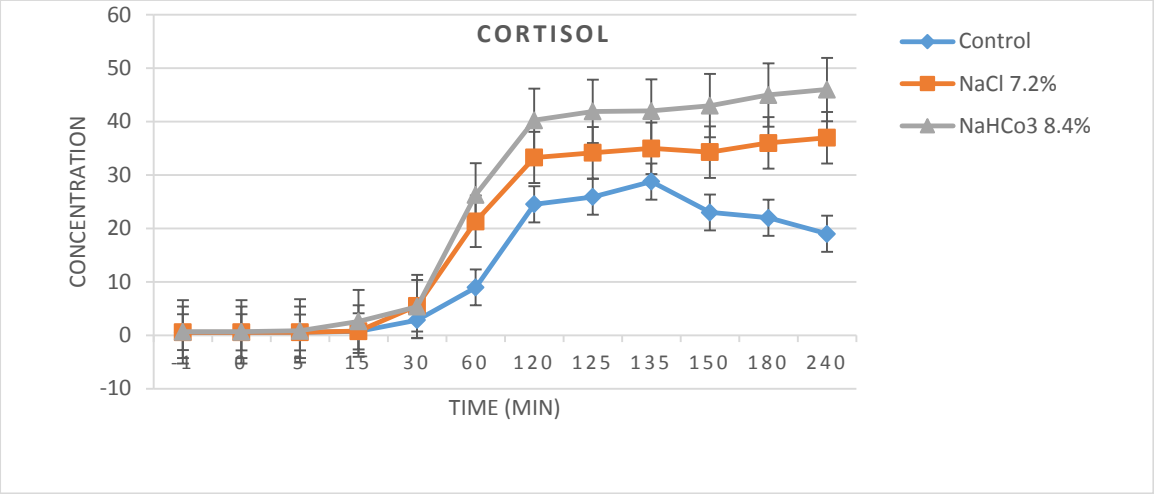

Fig 7: Mean (±SE) serum cortisol concentration of calves at each sampling time before and after injection of lipopolysaccharide (LPS) in control (NaCl 0.9%) and treatment (NaCl 7.2% and NaHCo3 8.4%) groups. Significant differences were found (P < 0.05).

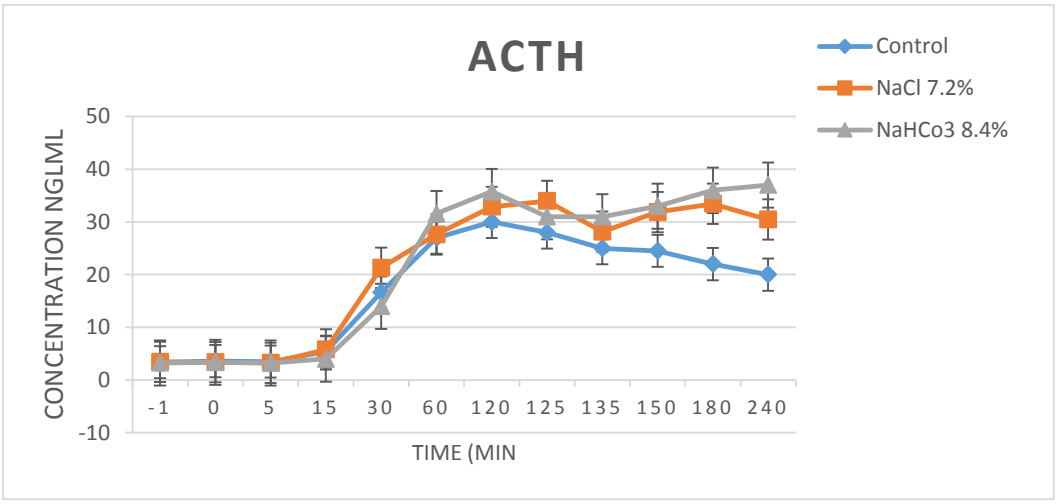

Fig 8: Mean (±SE) serum adrenocortico trophic hormone concentration of calves at each sampling time before and after injection of lipopolysaccharide (LPS) in control (NaCl 0.9%) and treatment (NaCl 7.2% and NaHCo3 8.4%) groups. Significant differences were found (P < 0.05).

Discussion

Endotoxemia is a common cause of morbidity and mortality of newborn calves which happens after infection with gram negative bacteria. It has been shown that para enteral administration of LPS produces a clinical syndrome similar to gram negative septicemia in calves and laboratory animals (Templeton *et al*. 1988; Wray & Thomlinson 1979). The objectives of fluid therapy in

calves with endotoxaemia are to correct dehydration, acidemia and reduce serum D-lactate concentrations in the blood (Naylor et al. 2006). There are various kinds of fluid which are used for correcting fluid and electrolytes imbalances in the calves. Diarrhea in which calves lose their suckling reflex IV infusion of isotonic solution is recommended as the treatment of choice (Berchtold, 1999). However, this therapeutic approach is hard to handle and expensive. As an alternative, the rapid IV injection of hypertonic solutions has been suggested (Constable, 2002). In the present study, the clinical efficacy of IV administration of hypertonic saline solution and hypertonic bicarbonate solution (HBS) in the treatment of complications of LPS IV injection was investigated.

Endotoxemia can cause fever, malaise and death. Fever is a characteristic of endotoxin administration and febrile response has been used as an indicator of severity of response (Elssaser *et al.* 1996). In the present study, similar to others (Elssaser *et al.* 1996; Kumar *et al*. 2001), an increase in rectal temperature was noticed following IV administration of LPS which reached its highest level after 2 hours. The severity of rectal temperature rise following LPS administration was concentration-dependent. In addition, without any intervention, the normothermia may take place after 4 hours of endotoxemia (Elssaser *et al*. 1996; Kumar & Kumar Malik 2001); however, in this study, approximately 15 minutes after hypertonic fluid therapy, the RT declined, indicating appropriate effects of hypertonic solutions on reducing harmful effects of endotoxins. No increase in the heart rate was noticed, in our study, after LPS administration (Fig.3), although increased HR was previously reported in calves that received LPS IV injection (Albertini *et al*. 2002; Königsson *et al*. 2002). Responses of the calves to the handling may interfere with the heart beats and masks any effects of LPS; somehow handling increases the base of heart rate and no further increase by LPS is detected (Borderas *et al.* 2008). The effects of LPS on RR obtained in this study agree with those reported previously in calves injected with LPS (Ganheim *et al.* 2003; Hüsler & Blum, 2001). An increase in RR, after LPS injection, is indicative of endotoxemia (Borderas *et al*. 2008), and in the present study, following IV injection of hypertonic solutions a significant decline in the rate of respiration was observed after 10 minutes. In comparison with the other two groups, the

NaCl 7.2% group showed a reduction in RR at 150 min.

Hyperlactatemia represents one prominent component of the metabolic response to sepsis, and it was observed that gram-negative bacteria elicits significant alterations in energy and carbohydrate metabolism (Fong *et al.* 1990). Acute infection alters carbohydrate metabolism by increasing peripheral glucose uptake and utilization, hyperlactatemia, enhancing glucose production, depressing glycogenesis, glucose intolerance and insulin resistance (Mizock *et al*. 1998).

In the current study, hyperlactatemia (Fig. 4) occurred 30 minutes p.a which was in agreement with the previous findings (Moore *et al.* 1980; Morris *et al.* 1986); however, in their study, Gerros *et al.* (1995) (78 mg/dl, 20 µg/kg) showed a maximum level of lactate after 2 hours p.a and believed that the magnitude and duration of lacticemia in calves are LPS dose-related. In the present study, serum lactate dramatically declined right after treatment with the hypertonic solutions with significant decrease after hypertonic sodium bicarbonate 8.4% administration; whereas, the control group treated by Isotonic NaCl (0.9%) showed no remarkable decrease (Giri *et al*. 1990).

Low serum albumin and total protein concentration are usually present in acute endotoxemia. Decreased albumin and total protein concentrations are in response to increased capillary permeability (Tennant *et al*. 1972). In this study, serum total protein showed slight fluctuations, reaching the lower limit in all groups at 120min of LPS administration and thus significant changes in each group were recorded; however, similar to other studies no statistical differences were found among groups (Mokhberdezfouli *et al*. 2014). The albumin is known as a negative in acute phase protein, decreasing the total protein rapidly, but is related to magnification of dehydration and endotoxemia (Dolente *et al*. 2007; Mokhberdezfouli *et al*. 2014). Fibrinogen is an efficient inflammatory index in ruminants (Smith, 2015). Mokhber Dezfouli *et al*. (2014) demonstrated that fibrinogen increased immediately after experimental septicemia which is similar to findings of the present study. Hypertonic solutions caused significant drop in such protein; somehow the sodium bicarbonate, at 180 and 240 minutes, indicated much more beneficial results. It has been well shown that infusion of hypertonic saline solution resulted in the hypothalamus-pituitary adrenal axis activation as evidenced

by elevation of circulating concentration of ACTH and cortisol (Cudd *et al*. 1998). Stimulatory effect of hypertonic saline solution on ACTH secretion was also reported (Raff *et al*. 1989; Ritmaster *et al*. 1987). In the present study, cortisol and ACTH levels (Fig. 7, 8) increased 30 minutes after endotoxin injection and remained elevated up to 2 hours and this trend continued even after hypertonic sodium bicarbonate administration; however, in the control group, the concentration continuously decreased up to the end of the study (19±5 µg/dl). The study by Giri *et al*. (1990) indicated that the duration and magnification of cortisol is LPS dose-dependent in which, by 2.5 µg/kg, the duration remained elevated for 12 hours. In the current study, hypertonic sodium bicarbonate significantly increased the levels of cortisol and ACTH to 46 and 37 µg/dl, during 2 hours, respectively. Hypertonic solutions may stimulate the hypothalamic-pituitary axis to increase cortisol level (Annane. 2005). In a study by Cudd, *et al.* (1998), it was revealed that the Adrenocorticotropic hormone and cortisol alterations in response to hypertonic saline are related to prostaglandin synthase inhibition. Singh *et al*. (1998) demonstrated that combination of hypertonic saline with Flunexinmeglumin causes much more efficient resuscitation in endotoxemic calves. Experimentally, the use of hypertonic saline in sublethal E. coli endotoxemia was associated with a more effective cardiovascular response rather than equal volume of isotonic saline solution.

In conclusion, the present study demonstrated that, after endotoxemia, several biochemical parameters altered and administration of hypertonic solutions induced faster returning to physiological state in which the hypertonic Sodium bicarbonate showed significantly more resuscitating effects than hypertonic and isotonic saline. In this study, sublethal doses of LPS induced mild transient endotoxemia and in more severe cases further research by higher doses of LPS are recommended.

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