Effects of Rapid and Ultra-Rapid Fluid Resuscitation Guided by Blood Lactate Clearance Rate in Diarrheic Calves

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Effects of Rapid and Ultra-Rapid Fluid Resuscitation Guided by Blood Lactate Clearance Rate in Diarrheic Calves

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ABSTRACT. The assessment of the results of intravenous fluid treatment in diarrheal calves is difficult under field conditions. To determine the effects of ultra-rapid (1 h) and rapid (3 h) intravenous infusions with 0.9% NaCl and 1.3% NaHCO3 solutions at a dose of 60 ml/kg on lactate clearance (LC) in calves with diarrhea. Sixty calves, including a healthy control group (n=20) and a group of calves with diarrhea (n=40), were used. Diarrheic calves were divided into two groups (n=20) according to solution type, and each group was then divided into two equal subgroups according to the infusion rate (n=10). Clinical and laboratory inspections of diarrheic calves were performed pre- and post-infusion, and the healthy control group was examined once. LC rates were calculated in the 1 h and 3 h subgroups. Marked improvements in clinical findings related to dehydration were observed in all groups with neonatal diarrhea that were given intravenous infusions with 0.9% NaCl and 1.3% NaHCO3 solutions. End of the infusion, the LC of the 0.9% NaCl and 1.3% NaHCO3 solutions in the ultra-rapid groups was significantly increased by 36.4% and 31.8%, respectively. However, for rapid infusion of the same solutions, 13.6% and 31.8% increases were observed, but the differences were not significant. Under field conditions, the LC of L- and D-lactate varied with the infusion rate, and these variations were significant in ultra-rapid subgroups for both solutions. Further studies will be designed for fluid therapy in calves based on the calculation of LC.

Keywords: Calf diarrhea, blood lactate clearance, rapid infusion, sodium bicarbonate, sodium chloride.
INTRODUCTION

Major challenges in calf husbandry tend to occur in the neonatal period (0-28 day), and the primary problem during this period is calf diarrhea (Basoglu et al., 2004; Sen and Constable, 2013). The high level of morbidity and mortality in the neonatal period is associated with complex etiological factors as well as the inability to perform effective treatments and the radical application of fluid therapy (Abeysekara et al., 2007). Metabolic acidosis and dehydration are other common problems that require specific treatments. Under field conditions, dehydration and metabolic acidosis of calves with diarrhea are estimated based on clinical signs due to the lack of laboratory equipment (Nakagawa et al., 2007). Metabolic acidosis occurs through the loss of bicarbonate through feces, which reduces the excretion of H ions from the kidneys and the accumulation of organic acids, such as lactate in the blood (Lorenz, 2009). Several studies have been published on lactate and D-lactatemia in calves with or without diarrhea (Omole et al., 2001; Ewaschuk et al., 2003), whereas the literature published on lactate in humans has focused on biomarker properties in sepsis and the assessment of trauma situations (Gomez and Kellum, 2015; Lee and An, 2016; Dekker et al., 2017).

The main goal of intravenous (IV) fluid therapy in diarrheic calves is to replace fluids, electrolytes, buffer and energy deficits. The route, amount, type and the rate of application of IV fluids for calves can change or are not suitable under field conditions (Kaske, 1994; Berchtold, 1999). Berchtold (1999) also stated that rapid infusion may cause cerebral edema, pseudoanemia, and hypoproteinemia. However, Kasari and Naylor (1985) as well as Kasari (1990) and Constable (2003) have recommended isotonic crystalloids for dehydrated calves at a maximum dose of 80 ml/kg/hr by an intravenous route. Another study found that an infusion rate of 80 ml/kg/hr may lead to hypernatremia and recommended that the dose be as low as 30 or 40 ml/kg/hr (Grove-White, 2007). Nevertheless, in children with neonatal diarrhea, Nager and Wang (2010) revealed the favorable effects of 1-h rapid infusions of isotonic crystalloid solutions at a dose of 60 ml/kg.

Lactate clearance (LC) is a simple and inexpensive clinical parameter based on the measurement of lactate levels between two serial times (Jones et al., 2010). LC indicates kinetic alterations in anaerobic metabolism, tissue hypoxia and septic conditions (Nguyen et al., 2010). In recent years, research has focused on this biomarker to discover the effects of fluid therapy in trauma and sepsis patients (James et al., 2011; Yu et al., 2013; Ghneim et al., 2013; Lyu et al., 2015). Interestingly, lactate clearance has not been systematically studied in fluid resuscitation in calves with diarrhea.

In this study, we aimed to determine the efficacy or inadequacy of ultra-rapid (1 h) and rapid (3 h) intravenous infusions with 0.9% NaCl and 1.3% NaHCO3 solutions administered at a dose of 60 ml/kg on lactate clearance in clinically dehydrated neonatal calves with diarrhea.

MATERIALS AND METHODS

Animals and clinical examination

Sixty animals, including healthy (n=20) and diarrheic (n=40) Holstein calves with moderate dehydration from both sexes and 1-3 weeks of age, were enrolled in the study. The clinically healthy control group calves were selected from 4 different dairy cattle farms in Aydin province in the first three-week period of their lives and included in the study. Diarrheic calves in the first three weeks of age that had moderate dehydration were referred to the clinic of researcher and author Erdogan for examination and treatment. Healthy calves were animals that received no treatment or had no infection history, whereas diarrheic calves had a treatment history.

Moderate dehydration (6 - 8%) was determined according to predictors, including skin turgor, dry mucous membranes and eyeball recession of 2 mm (Sen and Constable, 2013). Along with these clinical findings, the diarrheic calves were divided into two major groups (n=20 in each group) for the isotonic 0.9% NaCl solution and 1.3% NaHCO3 solution. The major groups were divided into two subgroups (n=10 in each group) for comparison of the effects of rapid (3 hr) and ultra-rapid (1 hr) infusions. Pre-treatment (Pre) and post-treatment (Post) clinical (e.g. eyeball recession, skin turgor, ability to stand, muscle tone and rectal temperature) were recorded for each calf.
suckling reflex, and mucosal membrane moistness) and laboratory data (Lactate, and D-lactate) were recorded.

Infusion protocol and blood samples
Calves in both groups were weighed with an electronic veterinary scale (Vaw 300 4P, Turkey) before treatment. The amounts of fluid for rehydration for both the 0.9% NaCl and 1.3% NaHCO3 solution groups were calculated based on the dosage of 60 ml/kg b.w. Fluid resuscitation was performed for all diarrheic calves in the Vena jugularis intravenously (Braun: 16 G 2” 1.7×50 mm 196 ml/min Lot: 1G16258253, Germany). For each diarrheic calf, the calculated amount of total fluid was infused with an infusion pump (Biocare IP12, Shenzhen Biocare Bio-Medical Equipment Co. Ltd, China). By changing the infusion rate of the pump, the total of both fluids (0.9% NaCl and 1.3% NaHCO3) was infused ultra-rapidly (1 hr) and rapidly (3 hr) to all subgroups. Diarrheic calves were randomly selected for the subgroups of both isotonic crystalloid solutions.

Blood samples were collected in lithium heparin (BD, USA) and a heparinized syringe (Genject Ca2+LH-100 I.U., Turkey) for biochemical and blood gas analyses prior to treatment and post-treatment. Post-treatment blood samples were taken in all infusion groups at the end of the fifth minute after the infusion completed. Heparinized blood samples taken pre- and post-treatment were centrifuged, and the obtained plasma was frozen at -20°C in Eppendorf tubes until analysis. L-lactate concentrations were detected via a hand held portable blood gas analyzer (EPOC, UK) and D-lactate concentrations were determined with a spectrophotometric method and ELISA using commercial test kits (Biovision, USA).

Lactate clearance
Lactate and D-lactate clearance (percent) were calculated by the following equation described by Nguyen et al.(2004):

\[
\text{Clearance} = \frac{\text{(Lactate pre-treatment)} - \text{(Lactate post-treatment)}}{\text{Lactate pre-treatment}} \times 100
\]

A negative value indicates reduction or clearance of L-lactate or D-lactate, whereas a positive value indicates elevation of lactate or D-lactate pre- and post-treatment intervention.

Statistical analyses
The average (X), standard deviation (SD) and minimum-maximum values (Xmin-Xmax) of the analysis parameters for L-lactate and D-lactate were calculated for each sample interval in the groups. The distribution of numerical data was evaluated using a Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used for comparison of more than two groups in normally distributed parameters, and a Tukey test was used for post hoc comparisons. Nonparametric methods were used for the analysis of non-normally distributed data. A Kruskal-Wallis test with Bonferroni correction was applied for post hoc comparisons for the comparison of parameters in more than two groups. In calves with diarrhea, the T-test and Wilcoxon test were performed on normally and non-normally distributed parameters, respectively, for comparison of pre- and post-treatment values of dependent groups. P values less than 0.05 were accepted as statistically significant (p<0.05). SPSS 15.0 software was used to analyze the statistical data.

RESULTS
Calves treated with isotonic NaCl solution at a dose of 60 ml/kg in the ultra-rapid (1 hr) and rapid (3 hr) groups showed marked improvement in skin turgor, mucosal membrane moistness, and eyeball recession, whereas there was no significant improvement in suckling reflexes, which were reduced post-treatment. Seven of ten calves that received ultra-rapid infusion in the 0.9% NaCl group urinated post-treatment; among those treated with rapid infusion, three out of ten calves urinated (30%). In the ultra-rapid infusion (URI) group and rapid infusion (RI) group of 0.9% NaCl, six and five calves were able to stand by themselves, respectively. However, the remaining 9 calves from both 0.9% NaCl solution groups were unable to stand by themselves. The ultra-rapid infusion subgroup of calves that received the isotonic (1.3%) NaHCO3 solution showed marked improvements in dehydration-related clinical findings, mental status and suckling reflexes.
post-treatment. In the rapid infusion subgroup of the 1.3% NaHCO3 solution group, these clinical improvements manifested in the second hour of infusion, and the sucking reflex recovered more slowly than in the ultra-rapid infusion group. At the second hour of rapid infusion of 1.3% NaHCO3 solution, all calves urinated; for the ultra-rapid infusion group calves using the same solution, 80% of calves urinated at the end of the infusion. Calves in the 1.3% NaHCO3 solution ultra-rapid infusion subgroup were able to stand by themselves, and the rapid infusion group calves were also able to stand during the second hour of the infusion. Two calves in the 1.3% NaHCO3 URI subgroup had unstable walking. In all infusion groups of calves were rehydrated and the presence and severity of dehydration was clinically reduced.

| Group                  | n  | Body weight (kg) | Amount of solution (mL) | Infusion rate (mL/min) |
|------------------------|----|------------------|-------------------------|------------------------|
| 0.9% NaCl Sol. URI     | 10 | 29.0 ± 2.5       | 1742.4 ± 147.8          | 29 ± 2.4               |
| 0.9% NaCl Sol. RI      | 10 | 30.0 ± 2.4       | 1798.2 ± 144.5          | 10 ± 0.8               |
| 1.3% NaHCO3 Sol URI    | 10 | 31.4 ± 3.2       | 1886.4 ± 193.2          | 31 ± 3.2               |
| 1.3% NaHCO3 Sol RI     | 10 | 30.2 ± 1.8       | 1813.2 ± 108.0          | 10 ± 0.6               |

There were no significant alterations in the amount of either ultra-rapid or rapid infusions of 0.9% NaCl solution and 1.3% NaHCO3 solution, as shown in Table 1.

L-lactate concentrations were statistically significantly higher for the pre-treatment 0.9% NaCl solution URI, 1.3% NaHCO3 solution URI and 1.3% NaHCO3 solution RI groups than for the control group calves. In the pre-treatment and post-treatment comparisons, ultra-rapid infusion and rapid infusion of both 1.3% NaHCO3 solution and 0.9% NaCl solution resulted in elevations in lactate concentration; these elevations were significant for ultra-rapid infusion of the 1.3% NaHCO3 and 0.9% NaCl solutions. Marked increases in LC were detected in the 0.9% NaCl solution URI, 1.3% NaHCO3 solution URI and 1.3% NaHCO3 solution RI groups. The 0.9% NaCl solution URI and 1.3% NaHCO3 solution URI group lactate clearance rates were 36.4% and 31.8%, respectively, and were statistically significant. There was no significant difference in D-lactate concentrations between the control group and the infusion groups of calves pre-treatment to post-treatment. In the URI and RI groups that received 0.9% NaCl solution, the D-lactate clearance increased, but the D-lactate clearance was reduced in the URI and RI groups that received the 1.3% NaHCO3 solution (Table 2).

**DISCUSSION**

Research has shown that lactate is a product of anaerobic glycolysis, and the production and clearance of lactate is balanced in body fluids. The daily production of lactate occurs in skeletal muscle, the brain, cardiac muscle, intestines, the renal medulla and erythrocytes; however, it is produced in almost all tissues and organs during disease (Sharkey and Wellman, 2015). The assessment of lactate concentrations has been used as a significant therapeutic and prognostic biomarker for human and animal health for many years (Nguyen et al., 2004; Karagiannis et al., 2006; Figueiredo et al., 2006; Allen and Holm, 2008; Zacher et al., 2010; Tennent-Brown et al., 2010). Lactate is commonly used to determine sepsis and its severity (Gomez and Kellum, 2015; Lee and An, 2016) as well as for the prognostic evaluation of patients with sepsis (Yu et al., 2013; Lyu et al., 2015). For example, in horses with acute abdomen, the influence of perioperative plasma lactate concentrations on the surgical intervention and recovery periods has been shown to be especially important (Donawick et al., 1975; Moore et al., 1976; Genn and Hertsch, 1982), and similar results have been observed in dogs with gastric dilatation and volvulus (De Papp, 1999). The
The importance of lactate has emerged in studies that were performed in association with lactic acidosis of the acute and subacute rumen (Ewaschuk et al., 2005) and in diseases, such as abomasum displacement and respiratory tract infections in cattle (Coghe et al., 2000; Figueiredo et al., 2006). Research evaluating variations in lactate levels (Kaske, 1994) and non-metabolized D-lactate levels (Lorenz and Gentile, 2014) in neonatal calves with diarrhea has appeared. It has been shown that lactate values can be used to determine the condition of strong ion acidosis (SID) in calves with diarrhea (Ewaschuk et al., 2003; Müller et al., 2012). However, no studies in the literature have evaluated lactate measurement or the effect of rehydration on lactate concentrations in calves with diarrhea. In this study, the influence of 0.9% NaCl and 1.3% NaHCO3 infusion solutions with a constant dose of 60 ml/kg on the pre-and post-treatment L-lactate and D-lactate concentrations was determined in moderately dehydrated calves with diarrhea. This is the first study comparing the effects of infusion rates of two isotonic solutions on lactate concentration and lactate clearance.

Over the last decade, it has been demonstrated that the result of a single lactate measurement can be contradictory for the interpretation of tissue perfusion (Zacher et al., 2010). Recently, research has indicated that conditions of cellular hypoxia and shock in humans must be evaluated over lactate clearance by measuring lactate concentrations at certain intervals (Vincent et al., 1983; Nguyen et al., 2004; Kamolz et al., 2005). In a study assessing the infusion rates of isotonic crystalloid and colloidal solutions of lactate clearance in patients with trauma, it was reported that the hydroxyethyl starch (HES) colloidal solution had significantly decreased lactate clearance compared with that of saline solution and resulted in less renal damage in patients with penetrating trauma; however, the relevant solutions did not cause any variation in the specified parameters in patients with blunt trauma (James et al., 2011).

In a study evaluating patients with sepsis who were admitted to an emergency clinic, different treatment groups underwent an increase in the central venous pressure (CVP) to 8 mmHg or greater. Overall, 4.5 L of isotonic crystalloid solution were administered on average for 6 h in a patient group, and the sepsis was treated. Because the study results were based on variations in sepsis levels and were not obtained by evaluating the patients who received infusions and different therapy methods, the efficacy of bolus fluid therapy on the lactate clearance could not be determined in the study (Jones et al., 2010). In another study, an average dose of 3,375 ml of crystalloid fluid altered lactate clearance at levels of 38.1% ± 34.6 and 12.0% ± 51.6 in surviving and non-surviving patients with sepsis, respectively (Nguyen et al., 2004). In our study, the ultra-rapid infusion (1 h) and the rapid infusion (3 h) of 0.9% NaCl solution at a dose of 60 ml/kg reduced the rates of lactate clearance to 36.4% and 13.6% in the calves, respectively. During ultra-rapid and rapid administration of 1.3% NaHCO3 solution to eliminate the dehydration as well as metabolic acidosis in calves with diarrhea, the variations in lactate clearance levels reduced to 31.8% in both

### Table 2. L and D-lactate concentrations and clearances levels in calves.

| Parameter | Control | 0.9% NaCl Sol. URI | 0.9% NaCl Sol. RI | 1.3% NaHCO3 Sol URI | 1.3% NaHCO3 Sol RI |
|-----------|---------|-------------------|-------------------|---------------------|-------------------|
| L. Lactate (mmol/L) | | | | | |
| Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| 0.9 ± 0.6 | 2.2 ± 1.7* | 3.0 ± 2.3† | 1.4 ± 1.0 | 1.4 ± 1.3 | 4.4 ± 2.8* | 5.8 ± 4.2* | 2.2 ± 2.1* | 2.9 ± 3.2* |
| D-lactate (mmol/L) | | | | | |
| 0.4 ± 0.1 | 0.41 ± 0.02 | 0.43 ± 0.13 | 0.39 ± 0.03 | 0.42 ± 0.14 | 0.39 ± 0.04 | 0.38 ± 0.05 | 0.41 ± 0.05 | 0.39 ± 0.06 |
| L. Laktat clearance | | | | | |
| Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| 36.4%* | 13.6%† | 31.8%* | 31.8%† |
| D-lactate clearance | | | | | |
| 4.9% | 7.7% | - 2.6%↓ | - 4.9%↓ |

*, †, #: Statistically significant from control group p<0.05, p<0.01 and p<0.001, respectively. 
↑: Increase (Reduce in Lactate Clearance) ; ↓: Decrease (Increases in Lactate Clearance); alterations in bold are statistically significant.

a,b: Different letters are statistically significant between pre and post treatment.
subgroups; however, the variations in the lactate concentrations were statistically significant in the ultra-rapid infusion group (Table 2). The relevant variations in lactate clearance were similar to those in human studies. The statistically significant reduce that occurred during ultra-rapid infusion of both solutions was considered to be due to the non-metabolizing of lactate that occurred within an hour despite eliminating dehydration and microcirculation. Furthermore, lactate clearance is closely related with capillary perfusion independent of hemodynamic variables (De Backer et al., 2006). The results in this study suggest that reducing in LC might be related to insufficient perfusion restoration, intravascular volume, and aliment energy metabolism. The outcome of statistically non-significant reduces in the clearance during ultra-rapid infusion was correlated with measurements in the third hour following fluid administration. In humans research based on the LC the sequential measurements of lactate is based on the least 6 hours intervals (Nguyen et al., 2004). In this study the time intervals between two lactate measurements were 3 hours. It is thought that the measurement of lactate clearance in such a short period of time is a limiting factor in the study.

Acute diarrhea in calves mainly causes dehydration, metabolic acidosis, electrolyte imbalance, prerenal azotemia and negative energy balance, which are independent of its etiology (Kaske, 1994; Berchtold, 1999), and these variations occur at different levels according to the duration and severity of diarrhea (Smith, 2009). Metabolic acidosis and dehydration are among the most significant complications of diarrhea (Smith, 2009). Additionally, metabolic acidosis may vary by age (Naylor, 1989). Trefz et al. (2012) found that median concentrations of D-lactate were detected at 0.8 mmol/l and 3.9 mmol/l in neonatal calves with diarrhea (age <7 days) and calves older than 7 days, respectively, and the age-dependent role of D-lactate was revealed from the development of metabolic acidosis. In this study, the level of D-lactate was in the range of 0.39-0.41 mmol/l in neonatal calves with diarrhea, and the contribution of D-lactate remained limited in the development of metabolic acidosis.

Although there are studies that have found associations between the elimination of D-lactate from the body and the clinical findings in calves (Lorenz, 2004), there are no studies regarding the association of D-lactate with renal clearance (Zello et al., 2008). In the study by Zello et al. (2008) the mean renal clearance was detected as 7.4 ± 3.4 ml/min when the blood concentration of D-lactate reached maximum levels in the calves receiving a D-lactic acid infusion. In the present study, the variations associated with infusion rates of the D-lactate concentration were observed in the blood, and the variations were measured at levels of 4.9% and 7.7% during the ultra-rapid and rapid infusions of 0.9% NaCl solution, respectively. On the other hand, variations were detected at levels of 2.6% and 4.9% during ultra-rapid and rapid infusion of 1.3% NaHCO3 solutions, respectively. In the calves treated with both infusion solutions, the low detectable concentrations of D-lactate, the low clearance of D-lactate in the blood and the low variation rates might be associated with the type of metabolic acidosis in calves with acute primary metabolic acidosis.

Several studies on different species have been conducted in the fields of human and veterinary medicine regarding the infusion rates of isotonic crystalloid solutions (Nager and Wang, 2010); however, the data that were obtained could not enhance routine practices. Because rapid infusion has risks, including hyperhydation and pulmonary edema, it has been suggested that the infusion rate should not be high. In addition to these side effects, cerebral edema, anemia and hypoproteinemia might also develop (Abeysekara et al., 2012). The maximum infusion rate of isotonic solutions has been reported to be as high as 80 ml/kg/h in dehydrated calves (Kasari, 1990; Constable, 2003). Grove-White (2007) found this rate to be risky in their study, and they argued there should be a lower infusion rate due to the risk of hyperhydration; they suggested lowering the rates to 30 or 40 ml/kg/h for a less risky and suitable infusion. In a study performed by Roussel and Kasari (1990), the best infusion rate was reported as 50 ml/kg/h in which the infusion of fluid was completed within 2 to 3 h, and the risk of hyperhydration was therefore eliminated. In present study, both 0.9 NaCl% and 1.3% NaHCO3 solutions were applied at 60 ml/kg/h for ultra-
rapid infusion and 20 ml/kg/h for rapid infusion in neonatal calves with moderate diarrhea. At the end of the infusion, the clinical findings for ultra-rapid and rapid infusions of isotonic NaCl solution in both calf groups with diarrhea were associated with an exacerbation of metabolic acidosis according to the relevant solution instead of the infusion rate.

CONCLUSION

The clearance of L-lactate and D-lactate varied with the infusion rate in calves with diarrhea, and these variations were statistically significant in both of the ultra-rapid subgroups. This study offers insight for further research on fluid therapy in calves based on the calculation of lactate clearance.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare for this manuscript.

Fig 1. Lactate concentrations in infusion groups

Fig 2. D-Lactate concentrations in infusion groups

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