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Evaluation of the optimal strong ion difference concentration of an oral electrolyte and buffering solution for the treatment of neonatal calf diarrhea

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Running head: TREATMENT OF NEONATAL CALF DIARRHEA

Abstract

An observational study was conducted to comparatively assess the efficacy of three different oral rehydration and buffering solutions, differentiated by their strong ion difference (SID) concentration, for treatment of neonatal calves with naturally acquired diarrhea. The SID concentrations tested were 100 mM, 170 mM and 230 mM for treatments SID_{100}, SID_{170} and SID_{230}, respectively. Clinical assessment and blood gas analysis were completed for 18 diarrheic calves once pre- and twice post- (6 and 24 hr after) oral administration with one of the three treatments. A repeated measure mixed model approach was used to analyze (a) the within-group efficacy of each treatment over time and (b) the between-group comparison at each timepoint. SID_{230} treatment resulted in a significant increase in blood pH, HCO_3^-, BE, SID and Na^+ at 6 and 24 hr after treatment, and a significant decrease in AG and K^+ by 24 hr after treatment. There were no significant changes in any of the blood gas parameters after treatment with SID_{100} and SID_{170}. SID_{230} treatment also resulted in blood gas parameter changes that were significantly different to the other two groups. These results suggest that the optimum SID concentration for the treatment of calves with diarrhea is likely to be higher than current recommendations.

Keywords: acidosis; neonatal calf diarrhea; oral electrolyte; strong ion difference

Introduction

Neonatal calf diarrhea (NCD) is the most common cause of morbidity and mortality in young calves [2, 28], accounting for over 50% of unweaned dairy heifer deaths [13,31]. Effective management and treatment of diarrhea is of crucial importance to animal health and welfare, as well as to the economics of an operation. An oral rehydration and buffering solution (ORBS) is the most common therapeutic intervention for calves suffering from NCD, particularly in cases with mild dehydration [9, 15]. An effective ORBS should be able to correct dehydration, electrolyte imbalance and acidosis [23].

Although the importance of an alkalinizing agent in an ORBS has been well established [4,16,17], the importance of an effective strong ion difference (SID) of an ORBS was first identified in 1996 [26]. The SID of a solution is a measurement of its alkalinizing ability and therefore its ability to correct metabolic acidosis which is a common occurrence in calves with diarrhea. Since establishment of the SID theory by Stewart [27], and its refinement by Constable [6], this principle has become a valid approach in formulating and evaluating the efficacy of treatments for calves with diarrhea and associated metabolic derangements [3, 25].

It is generally recommended that therapeutics for correcting electrolyte imbalance and acidosis in calves must record a minimum SID of 60 mM [5, 24], however, an optimum SID value for an ORBS treatment in diarrheic calves has not been fully scientifically evaluated.

The aim of this study was to examine three ORBS SID concentrations with a view to establish an optimal SID for treatment of acidosis in calves during a naturally occurring diarrheic episode. Our hypothesis was that the optimal SID concentration for enabling rapid and effective recovery from NCD and metabolic acidosis is higher than current recommendations.

Materials and Methods

An observational study of 18 calves, aged between 16 and 23 days, during diarrhea outbreaks was undertaken on three dairy farms in the Republic of Ireland. The calves from all three farms were Friesian/Friesian-cross male and female calves, housed in group housing with straw bedding. As per routine farm management, all calves received 3 l of colostrum via esophageal tube within 1 hr of birth

and were fed milk or milk replacer at a daily allowance of 6 l thereafter. The calves were enrolled in the study as soon as diarrhea (loose feces) was noticed by the farmer and all the calves with diarrhea were included in the study until the desired sample size was achieved.

The sample size was determined on the basis of an a priori power analysis (G-Power version 3.1.9.2) using blood pH data obtained from Sayers et al. [20]. This yielded a statistical power of 96% with a sample size of 6 animals per group to detect an expected 0.115 unit change in blood pH after treatment (difference between two dependent means), with a standard deviation of 0.07.

The calves were clinically assessed by two veterinarians, and by consensus, each calf was assigned a clinical assessment score (CAS) as per Sayers et al. [20]. In brief, clinically healthy calves were assigned a CAS of 0, with varying degrees of ill-health scored in increments of 1, to a maximum of 4 (0=healthy; 1=mild; 2=moderate; 3=severe; 4=grave), taking into account the following clinical parameters: calf demeanor, ear position, mobility, suckle reflex, enophthalmos, interest in surroundings and appetite. A CAS for each case calf was also recorded at blood sampling, 6 hr and 24 hr post-ORBS treatment.

Each case calf was blood sampled pre- and twice post-treatment by jugular venipuncture using 21G needles. The blood sample was taken 6 hr and 24 hr after the first ORBS administration. Samples were collected into heparinized syringes for the purposes of rapid blood gas analysis using a Siemens Rapidpoint 500 (Cruinn Diagnostics, Dublin, Ireland). Blood parameters reported by the analyzer included pH, bicarbonate ion ($HCO3^-$), base excess (BE), strong ion difference (SID), anion gap (AG), sodium (Na^+), potassium (K^+), chloride (Cl^-), hemoglobin (Hb) and glucose. Fecal samples were not obtained as part of this study and the cause of diarrhea was not investigated.

Calves were sequentially assigned to one of three ORBS treatment groups of varying SID concentration, in the order of SID_{100}, SID_{170} and SID_{230}. Treatment group SID_{100} (n=6; 4 male and 2 female, mean age 18 days), SID_{170} (n=6; 4 male and 2 female, mean age 20 days) and SID_{230} (n=6; 2 male and 4 female, mean age 19 days), received an ORBS with a SID concentration (and osmolarity) of 100 mM (667 mOsm/l), 170 mM (810 mOsm/l) and 230 mM (929 mOsm/l), respectively. SID was

calculated based on the following formula: [Sodium] + [Potassium] – [Chloride], and osmolarity was calculated using the following formula: 2x [Sodium Chloride] + 2x [Potassium Chloride] + 2x [Sodium Bicarbonate] + [Glucose]. The treatment solutions were formulated in-house using the required quantities of analytical grade glucose, bicarbonate, sodium, potassium and chloride, to yield the desired SID concentration. The detailed composition of the ORBS solution is presented in Table 1. Treatments were prepared as a powdered-based mixture which was reconstituted into 2 l of warm water immediately prior to administration. The treatments were administered twice, at timepoint 0 and 12 hr later, using an esophageal tube, as per usual practice on the farms in this study. Throughout this study all calves were fed milk or milk replacer at a daily allowance of 6 l and had unrestricted access to water.

Assessment of the effect of each treatment over time, comparing the within-group change between the pre- and the two post-treatment blood gas and CAS values was undertaken using a repeated measure mixed model approach. In total, 11 models were generated for the variables pH, $HCO3^-$, BE, AG, SID, Na^+, K^+, Cl^-, Hb, glucose and CAS score. Each model accounted for the effects of time (0 hr, 6 hr and 24 hr), the within-subject effects of treatment (SID_{100}, SID_{170} and SID_{230}), calf sex and calf identity nested within treatment, in addition to the interaction effects between treatment and time. Secondly, a between-group comparison analysis was undertaken to assess the unit change difference between the three groups at each timepoint. In order to standardize the group comparison and eliminate any pre-treatment (baseline) group effect, the pre-treatment values for all variables were subtracted from subsequent analyses. Eleven models were generated, each accounting for the effects of treatment (SID_{100}, SID_{170} and SID_{230}) and calf identity nested within treatment, the within-subject effects of time (0 hr, 6 hr and 24 hr), in addition to the interaction effects between treatment and time. In both cases, preliminary investigations into the most appropriate covariance structure for the final models (unstructured, autoregressive (first order) or exchangeable), based on the lowest value of the Akaike's and Schwarz's Bayesian information criteria, determined that auto-regressive (first order) structure was the most appropriate. Normality of the residuals was confirmed through kernel density estimate plots. Post-hoc pairwise comparison estimations of each final model were conducted, and post-

estimate predictions calculated, and reported where statistically relevant. Data management and graphical representations were completed using Microsoft Excel (Microsoft Office 2013, Microsoft Corporation, Redmond, WA, USA). All further statistical analyses were performed using Stata/SE v12.1 (StataCorp, College Station, TX, USA).

This study was conducted according to the guidelines of the Declaration of Helsinki, approved by the Teagasc Animal Ethics Committee (TAEC 81/2014 and TAEC 2020/264) and all procedures were authorized and carried out in accordance with the Health Products Regulatory Authority of Ireland (AE19132/P037 and AE19132/P120).

Results

Group mean blood gas and CAS values for pre- and post-treatments are presented in Table 2. Within-group analysis of the blood gas variables indicated that the SID_{230} treatment significantly increased the pH, HCO_3^-, BE, SID, Na^+ and Cl^- values, whereas the AG, K^+, Hb and CAS decreased significantly. The SID_{170} treatment led to a significant increase in Hb and a significant decrease in CAS after 24 hr. Treatment with SID_{100} did not result in any significant blood gas changes over time but the CAS improved significantly after 24 hr.

The rate of change in blood gas parameters and clinical improvement differed between treatment groups, as presented in Fig. 1. After SID_{230} treatment, the pH, HCO_3^-, BE, SID and Na^+ was significantly higher after 6 and 24 hr, when compared with SID_{100} and SID_{170} treatment. In comparison to group SID_{100} and SID_{170}, K^+ was significantly lower at 24 hr after SID_{230} treatment. There was a significant difference in AG between group SID_{100} and SID_{230} at 6 hr after treatment and between SID_{170} and SID_{230} at 24 hr after treatment. In terms of clinical improvement, it was significantly better for group SID_{230} at 6 hr after treatment in comparison to SID_{100} and SID_{170} treatment and at 24 hr when compared to SID_{100}. There was also a significant difference in clinical improvement between group SID_{100} and SID_{170} at 24 hr after treatment. Hb changes were significantly different for group SID_{170}, when compared to the other two groups at both timepoints. Furthermore,

there was a directional difference, where the Hb levels of group increased after SID_{170} treatment, whereas they decreased after the other two treatments.

All calves made a full recovery and continued to thrive two months after the study was completed, however two of the calves from SID_{100} group required an additional administration of the ORBS.

Discussion

The SID theory was initially proposed 40 years ago, and despite research efforts in certifying this theory, the optimal SID concentration range of an ORBS has not yet been established. The continued high mortality of neonatal calves with diarrhea is a major concern and the aim of this study was to attempt improving existing treatment options for this disease.

In this study, calves treated with an ORBS with a SID concentration of 230 mM demonstrated a more rapid normalization of blood gas variables and improvement in clinical health, relative to calves treated with the other two treatments of lower SID concentration. SID_{230} treatment was able to normalize the blood pH within 6 hr (after one treatment with 2 l of the ORBS), whereas SID_{170} treatment achieved this in 24 hr (after two treatments) and SID_{100} failed to achieve normal blood pH after 24 hr. The increased ability of SID_{230} treatment to correct metabolic acidosis is also confirmed by a significant increase in HCO_3^- and BE when compared to the other two groups. It would have been useful to determine the blood gas status more frequently and over a prolonged period of time, which is one of the limitations of this study. Previous studies have confirmed the efficacy of ORBS with a SID concentration >100 mM [20, 21, 25], however, Sen et al. [21] reported no significant differences when comparing an ORBS with a SID of 150 mM and 300 mM. More recent studies [33,35] evaluated the efficacy of different ORBS with SID concentrations in the range of 74-83 mM and although they report a comparative improvement in the acid-base status of the treated calves, it should be noted that, relative to the current study, the treatments were carried out over several days [33] and the calves were not acidaemic (mean blood pH value above lower reference limit) before the treatment [35].

High osmolarity in an ORBS is reported to have negative effects in terms of decreasing abomasal emptying and delaying plasma expansion [12, 18, 22]. In these studies the high osmolarity was mainly driven by the high glucose concentration, while the ORBS used in the current study all had a high osmolarity (and would be considered hypertonic), the hyperosmolarity was driven mainly by the high sodium content, particularly in the case of treatments SID_{170} and SID_{230}, with the glucose concentration remaining the same across all treatment groups. None of the calves examined here, receiving a solution with high osmolarity, showed clinical signs of abomasal ileus. This is consistent with the theory that delayed abomasal emptying is caused by hyperosmolarity influenced by glucose rather than ions (mainly sodium) which would question the benefit of increased glucose content [10]. In the current study, the glucose-to-sodium ratio of the treatments yielding beneficial effects (SID_{170} and SID_{230}) was lower (< 1.0) than current recommendations [24]. Previous studies using ORBS with a high SID and glucose-to-sodium ratio below 1.0 [21], or without any glucose [25], have also shown a significant improvement of the acid-base status of these calves thereby testing the theory of a benefit to an elevated glucose content in an ORBS. Additional studies are needed to determine the optimal glucose-to-sodium ratio of an ORBS for treatment of NCD [7]. Glucose and ions (particularly sodium) contribute to the osmolarity of an ORBS solution but have different effects on its tonicity [32], while only ions determine the SID concentration. Although the terms osmolarity and tonicity are often incorrectly used interchangeably, it may be more prudent to evaluate an ORBS efficacy and safety in terms of all three parameters.

Hypernatremia associated with administration of ORBS with high SID was a concern in this study. Cases of calves suffering from hypernatremia after inappropriate treatment have previously been reported [19, 34]. In the current study, the average sodium concentration of animals in the SID_{230} group 6 hr post-treatment was higher than recommended reference range of 133-140 mM [8] but it returned to normal values at 24 hr after treatment. In addition, the concentrations were below the threshold values for clinical hypernatremia in calves [1] and although the diarrheic calves treated with an ORBS with SID concentration of 230 mM had significantly higher blood concentration of sodium relative to the lower SID treatments, clinical signs of hypernatremia were not evident. Interestingly,

the sodium concentration in calves from group SID_{100} was higher than in group SID_{170} at 6 hr after treatment, but this difference was not significant. Chloride concentration increased significantly 6 hr after treatment with SID_{230}, although the ORBS chloride concentration was the same in all three groups. Although the chloride content in all three ORBS solutions was higher than current recommendation [23, 24], there was no significant increase after 24 hr in any of the groups. Under the conditions of the current study, no negative effects were observed. It is important to note that calves in this study had unrestricted access to drinking water, which would be crucial in reducing the risk of hypernatremia and hyperchloremia and improving the efficacy of this treatment. Unfortunately, it was not feasible to measure the water consumption in this observational study, as the calves were housed in groups with communal access to water. Milk consumption was also not evaluated, which is a limitation of this study. The impact of the feeding method was not assessed as part of this study, as all calves were stomach tubed, but could have also influenced the rate of absorption.

Surprisingly, a directional difference was detected in the level of hydration after treatment. Hemoglobin levels are considered a reliable guide for dehydration in calves with diarrhea [14] and they decreased after treatment with SID_{100} and SID_{230}. Values increased after treatment with SID_{170} group, however the hemoglobin levels of all calves in this group were lower in comparison to the other two groups before treatment. It has to be noted that all the hemoglobin levels were within the normal ranges and the calves in this study can only be categorized as mildly dehydrated [14]. Further research is required to evaluate the effect of high SID treatment on calves that are moderately or severely dehydrated.

Potassium was another variable to show a directional differentiation between the different groups and may highlight a potential biological mechanism that is associated with recovery. Evidence of a hypokalemic state for the SID_{230} treated animals in comparison to other groups could be associated with aldosterone and subsequent normalization of the acidosis [11, 29, 30]. Aldosterone stimulates distal tubular and collecting duct secretion of potassium in exchange for sodium and chloride ions, thereby inducing a (compensatory) metabolic alkalosis in calves with strong ion acidosis. It is possible that the high SID treatments (with a high sodium concentration combined with 4 l of water), may

enable and enhance the activity of aldosterone causing an increased renal excretion of potassium leading to hypokalemia, and absorption of sodium leading to hypernatremia. The significantly elevated sodium concentration in the SID_{230} group corroborates this potential mechanism.

This investigation has highlighted that the optimal SID for rapid correction of acidosis in diarrheic calves may be higher than current recommendations, but further research is required to determine the optimal SID concentration of an ORBS for treatment of NCD.

Conflict of interest

The authors declare there is no conflict of interest.

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Figure 1. Mean values for changes in blood gas variables and clinical assessment score over time, relative to pre-treatment values, in 18 calves following treatment with SID_{100} (⋯), SID_{170} (---) and SID_{230} (▬). The calves were administered the first treatment at timepoint 0 and the second treatment 12 hr later. [a,b] Letter superscripts not in common represent significant ($p < 0.05$) unit change difference between the treatment groups at each timepoint. Summary of the results of between-group comparisons is presented in Supplementary data.

Table 1. Composition of three oral electrolyte and buffering solutions (ORBS) as fed to calves.

	Treatment solutions		
Component	**SID_{100}**	**SID_{170}**	**SID_{230}**
Sodium (mM)	203	273	333
Chloride (mM)	129	129	129
Potassium (mM)	26	26	26
Bicarbonate (mM)	101	173	231
Glucose (mM)	205	205	205
Calculated SID (mM)	100	170	230
Calculated osmolality (mOsm/l)	667	810	929
Glucose: Sodium Ratio	1.01	0.75	0.62

SID = Strong Ion Difference

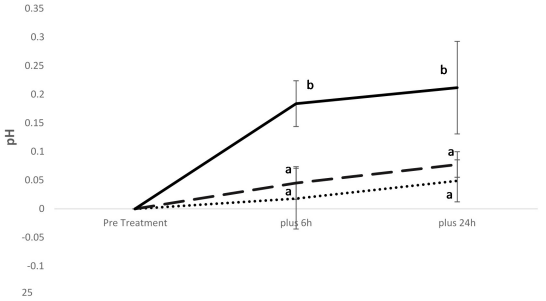
Table 2. Mean (SE) blood gas values and clinical assessment scores for 18 calves pre-treatment, 6 hours and 24 hours post-treatment with oral electrolyte and buffering solutions (ORBS) differentiated by their strong ion difference (SID) concentration (SID_{100}, SID_{170} and SID_{230}). Significant within-group differences over time, relative to pre-treatment values (timepoint 0 hr), are highlighted by astrixes, based on post-hoc estimates from repeated measures mixed models.

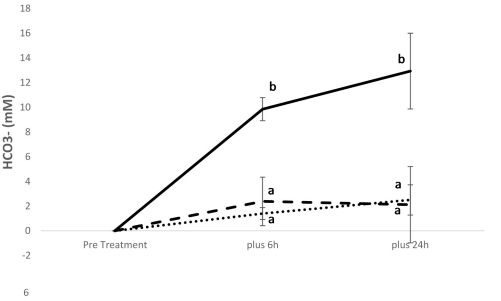
Variable	**Treatment Group**	**Timepoint 0hr (pre-treatment)**	**6hr (post-treatment)**	**24hr (post-treatment)**	**Reference range for healthy neonatal calves [8]**
pH	SID_{100}	7.299 (0.025)	7.317 (0.047)	7.348 (0.029)	7.373 – 7.466
	SID_{170}	7.304 (0.029)	7.350 (0.048)	7.395 (0.015)	
	SID_{230}	7.235 (0.046)	7.398 (0.027)***	7.414 (0.010)***	
HCO_3^- (mM)	SID_{100}	22.0 (1.7)	23.4 (1.7)	24.5 (2.0)	26.3–34.1
	SID_{170}	22.9 (1.9)	25.3 (2.2)	25.7 (1.4)	
	SID_{230}	19.2 (2.3)	28.2 (2.5)***	30.3 (2.1)***	
BE (mM)	SID_{100}	-2.4 (2.2)	-1.3 (2.5)	0.4 (2.8)	2.6–10.8
	SID_{170}	-2.1 (2.3)	0.4 (3.0)	2.0 (1.5)	
	SID_{230}	-7.0 (3.1)	4.2 (2.8)***	6.6 (2.2)***	
AG (mM)	SID_{100}	14.6 (0.8)	15.7 (3.3)	12.9 (1.5)	5.5–15.8
	SID_{170}	12.9 (1.3)	11.4 (1.1)	11.7 (1.2)	
	SID_{230}	15.8 (1.7)	14.8 (2.8)	10.8 (1.8)*	
SID (mM)	SID_{100}	38.4 (1.7)	39.6 (2.0)	39.7 (1.9)	39.4–48.8
	SID_{170}	37.2 (1.9)	37.2 (2.1)	39.2 (1.5)	
	SID_{230}	35.6 (2.0)	45.8 (1.7)***	43.2 (1.1)**	

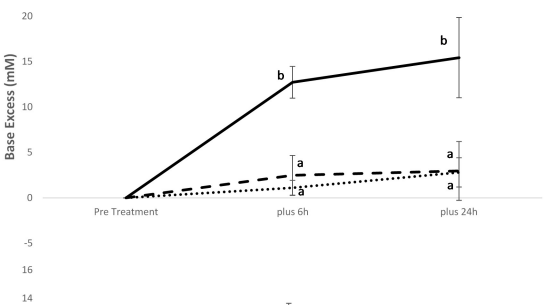
Na^+ (mM)	SID_{100}	135.8 (1.0)	138.5 (1.4)	137.3 (1.3)	133.3–140.2
	SID_{170}	134.6 (1.4)	134.0 (3.3)	137.3 (2.0)	
	SID_{230}	126.4 (3.1)	145.4 (6.2)***	137.2 (3.0)**	
K^+ (mM)	SID_{100}	4.60 (0.08)	4.57 (0.13)	4.67 (0.15)	4.13–5.41
	SID_{170}	4.53 (0.16)	4.31 (0.12)	4.68 (0.15)	
	SID_{230}	5.07 (0.26)	4.60 (0.31)	3.60 (0.31)***	
Cl^- (mM)	SID_{100}	102 (2)	104 (2)	102 (2)	93–101
	SID_{170}	102 (2)	101 (3)	103 (3)	
	SID_{230}	96 (1)	104 (5)**	98 (3)	
Glucose (mM)	SID_{100}	4.7 (0.7)	5.4 (0.7)	4.7 (0.5)	3.9 – 8.4
	SID_{170}	4.9 (0.3)	4.9 (0.7)	5.0 (0.4)	
	SID_{230}	4.8 (0.7)	4.3 (0.3)	5.3 (1.0)	
Haemoglobin (g/dL)	SID_{100}	11.75 (0.59)	10.8 (0.66)	11.08 (0.81)	8.6-14.3
	SID_{170}	8.73 (0.92)	11.73 (1.04)***	11.55 (0.85)***	
	SID_{230}	13.14 (0.51)	12.1 (0.10)	11.37 (0.47)**	
CAS	SID_{100}	1.5 (0.2)	1.3 (0.4)	0.8 (0.3)*	-
	SID_{170}	1.7 (0.3)	1.2 (0.4)	0.2 (0.2)***	
	SID_{230}	2.5 (0.3)	0.8 (0.3)***	0.7 (0.3)***	

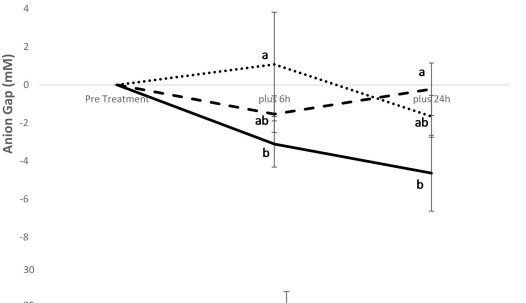
*P ≤0.05; ** P ≤0.01; *** P <0.001.

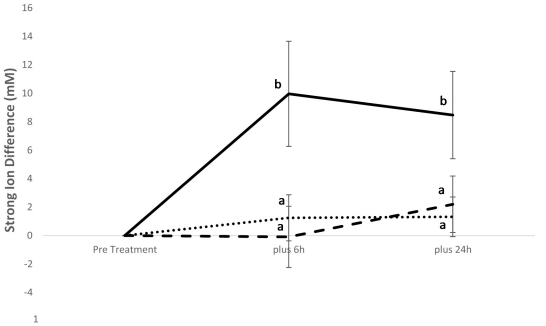
[8] Dillane et al., 2018

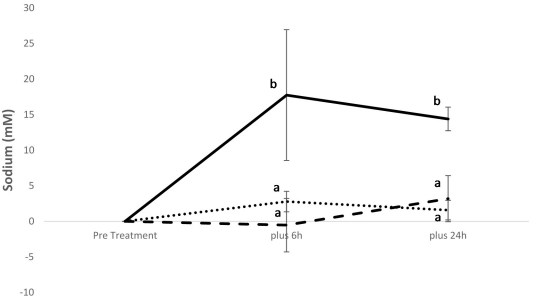

pH
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
Pre Treatment
plus 6h
plus 24h
b
b
a
a
a
a

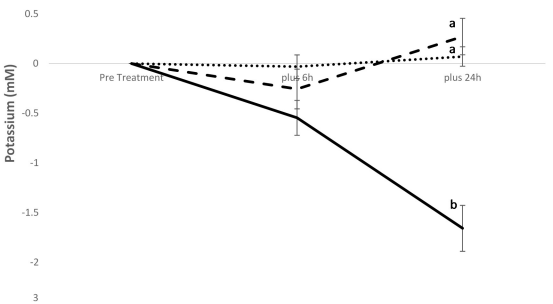

HCO3- (mM)
18
16
14
12
10
8
6
4
2
0
-2
Pre Treatment
plus 6h
plus 24h
b
b
a
a
a
a

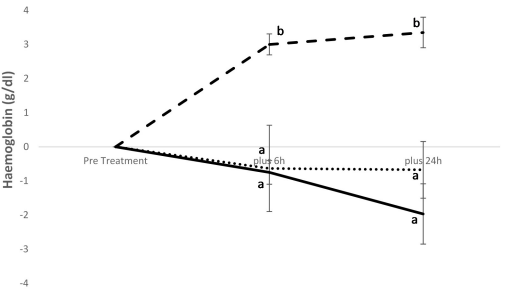

Base Excess (mM)
20
15
10
5
0
-5
Pre Treatment
plus 6h
plus 24h
b
b
a
a
a
a

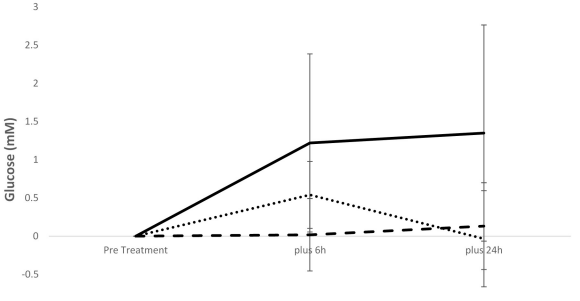

Anion Gap (mM)
6
4
2
0
-2
-4
-6
-8
Pre Treatment
plus 6h
plus 24h
a
a
a
ab
ab
b
b

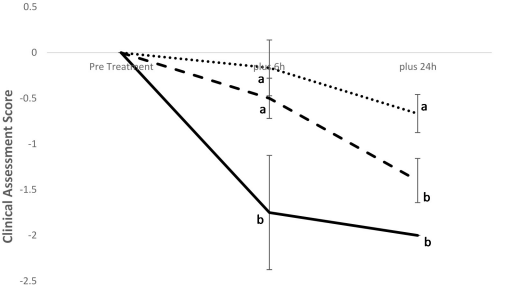

Strong Ion Difference (mM)
16
14
12
10
8
6
4
2
0
-2
-4
Pre Treatment
plus 6h
plus 24h
b
b
a
a
a
a


Sodium (mM)
30
25
20
15
10
5
0
-5
-10
Pre Treatment
plus 6h
plus 24h
b
b
a
a
a
a


Potassium (mM)
0.5
0
-0.5
-1
-1.5
-2
Pre Treatment
plus 6h
plus 24h
a
a
b


Haemoglobin (g/dl)
4
3
2
1
0
-1
-2
-3
-4
Pre Treatment
plus 6h
plus 24h
b
b
a
a
a
a


Glucose (mM)
3
2.5
2
1.5
1
0.5
0
-0.5
-1
Pre Treatment
plus 6h
plus 24h


Clinical Assessment Score
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
Pre Treatment
plus 6h
plus 24h
a
a
a
b
b

Supplementary Material

Supplementary Table 1

Pairwise comparisons of adjusted prediction output following repeated measures mixed model analysis. Contrast values reflect the unit change difference between the treatment groups at the two post-treatment timepoints, with all other variable held constant, for each blood gas variables and clinical assessment score.

Variable	Timepoint	Treatment Group	Contrast	SE (Delta method)	P value (unadjusted)
pH	+6 hr	SID170 vs SID100	0.003	0.038	0.47
		SID230 vs SID100	0.16	0.043	<0.0001
		SID230 vs SID170	0.14	0.043	0.002
	+24 hr	SID170 vs SID100	0.02	0.040	0.66
		SID230 vs SID100	0.16	0.047	<0.0001
		SID230 vs SID170	0.02	0.049	0.003
HCO_3^- (mM)	+6 hr	SID170 vs SID100	0.98	1.70	0.56
		SID230 vs SID100	7.98	1.90	<0.0001
		SID230 vs SID170	7.00	1.91	<0.0001
	+24 hr	SID170 vs SID100	-0.49	1.77	0.78
		SID230 vs SID100	10.12	2.08	<0.0001
		SID230 vs SID170	10.61	2.16	<0.0001
BE (mM)	+6 hr	SID170 vs SID100	1.38	2.05	0.50
		SID230 vs SID100	11.07	2.28	<0.0001

		SID230 vs SID170	9.69	2.29	<0.0001
	+24 hr	SID170 vs SID100	-0.12	2.14	0.96
		SID230 vs SID100	12.24	2.50	<0.0001
		SID230 vs SID170	12.35	2.60	<0.0001
AG (mM)	+6 hr	SID170 vs SID100	-2.62	1.57	0.10
		SID230 vs SID100	-4.47	1.79	0.01
		SID230 vs SID170	-1.86	1.80	0.30
	+24 hr	SID170 vs SID100	1.54	1.66	0.35
		SID230 vs SID100	-3.39	1.95	0.08
		SID230 vs SID170	-4.93	2.03	0.015
SID (mM)	+6 hr	SID170 vs SID100	-1.34	1.998	0.50
		SID230 vs SID100	8.33	2,25	<0.0001
		SID230 vs SID170	9.68	2.25	<0.0001
	+24 hr	SID170 vs SID100	0.87	2.08	0.67
		SID230 vs SID100	6.78	2.45	0.006
		SID230 vs SID170	5.90	2.55	0.02
Na^+ (mM)	+6 hr	SID170 vs SID100	-3.32	3.52	0.34
		SID230 vs SID100	14.55	4.00	<0.0001
		SID230 vs SID170	17.87	3.99	<0.0001

	+24 hr	SID170 vs SID100	1.06	3.69	0.77
		SID230 vs SID100	12.68	4.35	0.004
		SID230 vs SID170	11.62	4.52	0.01
K^+ (mM)	+6 hr	SID170 vs SID100	-0.19	0.15	0.20
		SID230 vs SID100	-0.24	0.17	0.16
		SID230 vs SID170	-0.05	1.17	0.77
	+24 hr	SID170 vs SID100	0.13	0.16	0.40
		SID230 vs SID100	-2.00	0.19	<0.0001
		SID230 vs SID170	-2.13	0.19	<0.0001
Cl^- (mM)	+6 hr	SID170 vs SID100	-2.16	2.58	0.40
		SID230 vs SID100	5.93	2.93	0.04
		SID230 vs SID170	8.09	2.94	0.006
	+24 hr	SID170 vs SID100	0.59	2.71	0.82
		SID230 vs SID100	3.88	3.19	0.22
		SID230 vs SID170	3.28	3.32	0.32
Glucose (mM)	+6 hr	SID170 vs SID100	-0.53	0.63	0.40
		SID230 vs SID100	0.43	0.72	0.54
		SID230 vs SID170	0.96	0.71	0.18
	+24 hr	SID170 vs SID100	0.21	0.66	0.75

		SID230 vs SID100	1.28	0.78	0.10
		SID230 vs SID170	1.07	0.81	0.19
CAS	+6 hr	SID170 vs SID100	-0.33	0.28	0.23
		SID230 vs SID100	-1.67	0.32	<0.0001
		SID230 vs SID170	-1.34	0.31	<0.0001
	+24 hr	SID170 vs SID100	-0.72	0.29	0.01
		SID230 vs SID100	-1.35	0.34	<0.0001
		SID230 vs SID170	-0.63	0.36	0.07
Hb (g/dL)	+6 hr	SID170 vs SID100	3.19	0.63	<0.0001
		SID230 vs SID100	0.06	0.69	0.93
		SID230 vs SID170	-3.14	0.71	<0.0001
	+24 hr	SID170 vs SID100	3.69	0.66	<0.0001
		SID230 vs SID100	-0.80	0.60	0.18
		SID230 vs SID170	-4.49	0.73	<0.0001