



J. Dairy Sci. 99:4647–4655

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## An observational study using blood gas analysis to assess neonatal calf diarrhea and subsequent recovery with a European Commission-compliant oral electrolyte solution

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### ABSTRACT

An observational study was conducted on dairy calves (51 healthy, 31 with neonatal diarrhea) during outbreaks of diarrhea on 4 dairy farms. Clinical assessment scores (CAS) were assigned to each healthy and diarrheic calf [from 0 (healthy) to 4 (marked illness)]. Blood gas analysis [pH, base excess (BE), Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>, glucose, total hemoglobin, standard HCO<sub>3</sub><sup>-</sup>, strong ion difference (SID), and anion gap (AG)] was completed for each calf. Repeated measurements were taken in healthy animals, and pre- and postintervention measurements were taken for diarrheic calves. The mean CAS of diarrheic calves was 1.7, with 51, 30, 17, and 2% of calves scoring 1, 2, 3, and 4, respectively. The mean value for blood pH, BE, AG, and SID was 7.26, -4.93 mM, 16.3 mM, and 38.59 mM, respectively. Calves were administered an oral rehydration and buffering solution (ORBS; Vitalife for Calves, Epsilon Ltd., Cork, Ireland) and reassessed. The mean CAS decreased to 0.38 (65% of calves scored 0 and 35% scored 1) at 6 to 18 h posttreatment and to 0.03 (98% of calves scored 0 and 2% scored 1) within 24 to 48 h. Significant increases in mean value for pH, BE, HCO<sub>3</sub><sup>-</sup>, Na<sup>+</sup>, and SID, and significant decreases in AG, K<sup>+</sup>, Ca<sup>2+</sup>, and total hemoglobin were recorded posttreatment. The correlation estimates indicated that pH, HCO<sub>3</sub><sup>-</sup>, and BE were strongly correlated with CAS, with values exceeding 0.60 in all cases. Administration of an ORBS with a high SID and bicarbonate buffer demonstrated rapid recovery from a diarrheic episode in dairy calves. **Key words:** acidosis, blood gas analysis, electrolyte, neonatal calf diarrhea

### INTRODUCTION

Neonatal calf diarrhea is the most common cause of mortality in calves (Torsein et al., 2011; Azizzadeh et al., 2012). Electrolyte disturbance, dehydration, and metabolic acidosis, accompanied by a strong ion difference (SID), are the most significant consequences of diarrhea in calves (Smith and Berchtold, 2014). Veterinary assessment of calves with diarrhea is generally based on clinical examination alone; however, blood gas analysis remains the most detailed approach to assess the degree of electrolyte disturbance and acidosis in diarrheic calves. Russell and Roussel (2007) have previously highlighted blood gas analysis as a useful tool in practice, especially when combined with history and physical examination.

In many cases, initial diagnosis and treatment of neonatal calf diarrhea is predominantly carried out by primary producers (farmer or manager), who utilize an oral rehydration and buffering solution (ORBS) as a first inexpensive attempt to address calf diarrhea. An ORBS is recommended for a diarrheic calf when dehydration is less than 8% and there is still evidence of a suckle reflex (Lorenz et al., 2011). The purpose of the ORBS is to promote plasma expansion, correct electrolyte imbalances, and provide glucose as a co-transport partner of sodium to facilitate water resorption and an alkalizing agent to address the strong ion or metabolic acidosis (Smith, 2009). However, uncertainty remains regarding the optimal electrolyte concentrations, type of buffer, energy source, and osmolality of the ideal ORBS solution (Naylor, 1989; Constable et al., 2009; Sen et al., 2009). Accordingly, a large number of ORBS products are commercially available, differentiated by composition and administration protocols (Smith and Berchtold, 2014). This makes it difficult for producers and veterinarians to identify a product that best suits the needs of diarrheic calves.

Received November 5, 2015.

Accepted February 13, 2016.

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European Commission regulation No 5/2014 amending European Directive 2008/38/EC (European Union, 2014) sets requirements and recommendations for an ORBS to be suitable for treatment of electrolyte imbalance and acidosis in calves. It emphasizes a minimum SID value of 60 mM for such therapies. Based on the interpretation of Stewart (1981), SID is regarded as the major factor in determining the alkalinity of an ORBS and as a valid approach when formulating an ORBS for calves with diarrhea and metabolic derangement (Stämpfli et al., 2012). The optimal SID for an ORBS has not been determined, with estimates ranging from 60 mM (Smith and Berchtold, 2014) to 110 mM (Stämpfli et al., 2012).

The question as to which is more important—an ORBS with high SID or an ORBS with an alkalinizing agent—has yet to be definitively answered (Smith and Berchtold, 2014), and no consensus exists on a suitable alkalinizing agent. The use of bicarbonate precursors such as acetate or propionate are favored over bicarbonate for their energy value once metabolized, their water absorption capabilities, and the fact that they do not alkalinize the abomasum. Bicarbonate was believed to inhibit abomasal milk clotting; however, this has not been supported by recent studies (Bachmann et al., 2009; Constable et al., 2009).

Unlike medicines, which undergo rigorous testing before European Commission (EC) approval, ORBS within Europe are not assessed for clinical efficacy before market placement. Although a limited number of studies have assessed various aspects of ORBS treatment in natural neonatal calf diarrhea (Naylor, 1989; Stämpfli et al., 1996, 2012; Constable et al., 2009; Grünberg et al., 2013; Kirchner et al., 2014), there is a lack of observational field studies in recent years examining the efficacy and suitability of ORBS for use in calf diarrhea (Meganck et al., 2014), particularly for data relating to ORBS conforming to recently amended European Union (EU) legislation.

In an attempt to increase scientific knowledge in this area, the aim of this observational study was to investigate outbreaks of calf diarrhea on 4 dairy farms using rapid “pen-side” blood gas analysis and subsequently evaluate treatment of diarrheic calves using an ORBS that is compliant with current EU legislation.

## MATERIALS AND METHODS

### Study Approval

This study was approved by the Teagasc Animal Ethics Committee (TAEC 81/2014); all procedures were authorized and carried out in accordance with

the Health Products Regulatory Authority (HPRA) of Ireland (AE19132/P037).

### Clinical Assessment Score

To comparatively assess diarrheic calves pre- and posttreatment, a 5-point clinical assessment scoring (CAS) chart was used. This chart was developed for use by farm managers and veterinarians at Teagasc (Irish Agriculture and Food Development Authority, Carlow, Ireland) dairy research farms. 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, as outlined in Supplementary Figure S1 (<http://dx.doi.org/10.3168/jds.2015-10600>). We constructed the chart based on previously published dehydration charts (Naylor, 1989) and the Wisconsin respiratory calf health-scoring model ([http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf\\_health\\_scoring\\_chart.pdf](http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf); McGuirk, 2008). The chart incorporated calf demeanor, ear position, mobility, suckle reflex, enophthalmos, and desire-to-feed variables. Temperature was not recorded, as the study sought to use variables most indicative of dehydration and metabolic acidosis, and variables that would be routinely observed by producers on commercial farms. Additionally, no attempt was made to identify the underlying cause of the diarrhea, as it was not the focus of the research. Clinical assessment was completed before each blood sample was taken and, in the case of diarrheic calves, an additional assessment was conducted at 24 to 48 h posttreatment. All calves were assessed and scored simultaneously by 2 research veterinarians and a single consensus score was recorded. All CAS were recorded before generation of blood gas results.

### Sample Population

An observational study of 77 calves from 2 research (A and B) and 2 commercial (C and D) dairy farms was completed over a 21-d period in spring 2015. A description of husbandry regimens on each study farm for calves in the first month of life is presented in Table 1. Calves were defined as clinically healthy if they recorded a CAS of 0 (as previously described) and had no evidence of diarrhea. Healthy calves were sampled on farm A during a period when no cases of diarrhea had been recorded on the farm from the start of the calving season to the time of assessment ( $n = 28$ ; 71 measurements). Healthy case animals were also identified on farms B ( $n = 4$ ; 6 measurements) and C ( $n = 19$ ; 19 measurements) during a period of diarrhea outbreak on those farms. Diarrheic case calves were defined as

**Table 1.** Description of calf husbandry regimens on each study farm for calves in the first month of life

Farm	Predominant calf breeds <sup>1</sup>	Housing	Shared airspace with adult cows?	Ad libitum water available?	Milk feeding system	Creep feed available?
A	HF, JeX	Individual calf pen followed by group pens (up to 12 animals) at 3 d of age. Deep straw bedding in all pens.	Yes	Yes	Manual multi-calf feeding buckets with an allowance of 6 L of milk replacer or whole milk per calf per day.	Yes, from 1 wk of age
B	HF	Individual calf pen followed by group pens (up to 25 animals) at 3 d of age. Deep straw bedding in all pens.	No	Yes	Automatic feeders with an allowance of 6 L of milk replacer per calf per day as a routine. Isolated and switched to manual feeding if diarrheic.	Yes, from 1 wk of age
C	HF, JeX	Deep straw-bedded group pens from birth (up to 20 animals).	No	Yes	Manual multi-calf feeding buckets with an allowance of 6 L of milk replacer per calf per day.	Yes, from 1 wk of age
D	HF, JeX	Straw-bedded group pens from birth (up to 10 animals), moving to woodchip bedded group pens (up to 20 animals) from approximately 2 wk of age.	No	Yes	Manual multi-calf feeding buckets with an allowance of 4 L of milk replacer per calf per day.	No

<sup>1</sup>HF = Holstein-Friesian; JeX = Jersey cross.

having a CAS of 1 or greater, and evidence of diarrhea. Such calves were identified on farms B (n = 9), C (n = 2), and D (n = 12). A diarrhea outbreak subsequently occurred on farm A that facilitated analysis of an additional 8 calves, 5 of which were sampled earlier as part of the healthy cohort. All animals, both healthy and diarrheic, were enrolled in the study between the ages of 7 and 26 d.

### Sampling and Administration of ORBS

Each case calf was blood sampled by jugular venipuncture on at least 1 but not more than 3 occasions over the duration of the study. Venous blood samples were taken into heparinized 1-mL syringes (Cruinn Diagnostics, Dublin, Ireland), immediately placed on a bottle roller, and continuously agitated for at least 20 s to avoid formation of microclots. Before testing, all visible air bubbles were expelled from the syringe. A bench-top Rapidpoint 400 (Siemens, Munich, Germany) analyzer was used to test all samples. Parameters reported by the analyzer included pH, base excess (**BE**; mM), Na<sup>+</sup> (mM), K<sup>+</sup> (mM), Ca<sup>2+</sup> (mM), Cl<sup>-</sup> (mM), glucose (mM), total hemoglobin (**Hb**; g/dL), standard HCO<sub>3</sub><sup>-</sup> (mM), and anion gap (**AG**; mM). For healthy calves, samples were taken over a period of 3 d, approximately 2 h after feeding. In the case of diarrheic calves, pretreatment samples were taken within 2 h of a milk feed being offered (many of the diarrheic calves

had diminished suck reflexes and either fed to a limited degree or not at all). These calves were then administered an ORBS (Vitalife for Calves; Epsilon Ltd., Cork, Ireland) reconstituted in water according to the manufacturer's instructions. The final formulation has a reported SID of >100 mM and includes a bicarbonate buffer. All treatments were administered by esophageal tube. Posttreatment blood samples were collected between 6 and 18 h following ORBS intervention.

### Additional Calf Data

Accurate calf date of birth, sex, breed, birth weight, whether the calf was a singleton or twin, and the level of calving difficulty experienced by the dam were available for all calves from farm A. On-going regular weight data (weekly or every 2 wk) were only available from farm A and included measurement on all 8 diarrheic calves, and 20 of the 23 healthy calves.

### Data Analysis

Data management and graphical representations were completed using Excel (Office 2010, Microsoft Corp., Redmond, WA). Preliminary steps established the stability of the variance for each of the continuous variables. For the purposes of analysis, results for calves recording CAS of 3 and 4 were grouped. Associations between various genetic and environmental fac-

**Table 2.** Mean blood gas values for healthy calves and diarrheic calves (pre- and post-administration of oral rehydration and buffering solution)

Blood gas variable	Healthy calves in a diarrheic-free environment (farm A), Value (SEM) (n = 28; 72 measurements)	Diarrheic calves	
		Pretreatment [range] (SEM) (n = 31)	Posttreatment [range] (SEM) (n = 31)
pH	7.42 (0.004)	7.26 [6.76, 7.39] (0.019)	7.42 [7.28, 7.49] (0.007)**
HCO <sub>3</sub> <sup>-</sup> (mM)	29.78 (0.311)	20.3 [6.1, 27.1] (0.793)	30.9 [16.3, 38.9] (0.763)**
Base excess (mM) <sup>1</sup> standard	6.00 (0.339)	-4.9 [-31.6, 3.3] (1.173)	7.3 [-10.3, 18.6] (0.857)**
Anion gap (mM) <sup>1</sup>	12.77 (0.405)	16.3 [9.2, 31.8] (0.865)	12.2 [5.1, 23.7] (0.583)*
Na <sup>+</sup> (mM)	138.94 (0.317)	135.0 [118.4, 158.5] (1.399)	143.1 [131.4, 186.2] (1.662)**
K <sup>+</sup> (mM)	4.85 (0.040)	4.89 [3.39, 6.59] (0.122)	4.32 [3.26, 5.51] (0.018)*
Cl <sup>-</sup> (mM)	99.56 (0.425)	101.3 [84, 120] (1.265)	101.9 [91.0, 134.0] (1.506)
SID <sup>2</sup> (mM)	44.23 (0.411)	38.59 [26.84, 52.18] (0.819)	45.53 [35.43, 56.92] (0.658)**
Glucose (mM)	7.91 (0.359)	5.2 [2.5, 8.3] (0.178)	5.5 [3.7, 8.8] (0.180)
Ca <sup>2+</sup> (mM)	1.26 (0.007)	1.27 [1.14, 1.42] (0.012)	1.20 [1.01, 1.33] (0.012)**
Total hemoglobin (g/dL)	11.67 (0.183)	13.5 [7.7, 20.0] (0.438)	12.1 [9.6, 15.7] (0.276)*

<sup>1</sup>Calculated using blood gas machine algorithm.

<sup>2</sup>Strong ion difference = [Na<sup>+</sup>] + [K<sup>+</sup>] - [Cl<sup>-</sup>].

\**P* = 0.001, \*\**P* < 0.0001: statistical difference between pre- and posttreatment values estimated using linear regression.

tors and blood gas measurements were investigated in the healthy calf cohort from farm A. A cross-sectional, time-series, generalized estimating equation (xtgee procedure; Stata, StataCorp LP, College Station, TX) was used to account for repeated measures. The model tested the combined effect of sex, calving difficulty, breed, date of calving, birth weight, and single or twin on each of the 11 blood gas variables (pH, HCO<sub>3</sub><sup>-</sup>, BE, AG, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, glucose, total Hb, and SID). Each model was constructed using a Gaussian distribution, identity link, and an exchangeable correlation. The effect of ORBS treatment on the 11 blood gas measurements of diarrheic calves was assessed by linear regression, with status (pre- and post-ORBS treatment) used as the independent variable. The effect of environment (diarrhea or diarrhea-free) on the 11 blood gas measurements of healthy calves was assessed by linear regression, with environmental status used as the independent variable. A further linear regression model was constructed to assess the effect of ORBS treatment on CAS. A Student *t*-test was used to comparatively assess calf weight at weekly, or every other week, weight measurement time points. A Spearman correlation was used to determine the association between each blood gas variable and CAS. To achieve this, the continuous blood gas variables were reclassified as ordinal data using the standard deviation value for each variable as an increment gap size. Each increment was ranked sequentially on an ordinal scale, with rank 1 as the lowest in each case. Finally, a Pearson correlation was used to determine the association between bicarbonate and SID. *P*-values of ≤ 0.05 were considered statistically significant. All statistical analysis was performed using Stata/SE v12.1 (StataCorp LP).

## RESULTS

The blood gas profile of diarrheic calves is presented in Table 2, with healthy calf values presented for comparative purposes. The treatment of diarrheic calves with an EC-compliant ORBS led to a significant increase in mean values (*P* < 0.001) for pH, BE, HCO<sub>3</sub><sup>-</sup>, Na<sup>+</sup> and SID relative to pretreatment diarrheic calf values, whereas a significant decrease (*P* < 0.001) was recorded for AG, K<sup>+</sup>, Ca<sup>2+</sup>, and total Hb. None of the 31 ORBS-treated animals died during the postmonitoring clinical assessment period of 8 d. On research farms A and B, where longer term records were maintained, all treated animals made a full recovery, as determined by CAS values of 0, and were returned to the general calf population from hospital facilities.

The blood gas results of healthy calves reared in a diarrhea-free environment and in a diarrhea environment are presented in Table 3. With 4 exceptions (bicarbonate, SID, BE, and AG), these results correspond with previously published reference ranges. Statistical comparisons between these 2 groups indicated that the calves reared in a diarrhea environment had significantly lower values for pH, AG, Na<sup>+</sup>, Cl<sup>-</sup>, and glucose.

The CAS for pre- and post-ORBS-treated diarrheic case calves is presented in Figure 1. The mean CAS for pretreatment diarrheic calves was 1.7, with 49% of cases recording a CAS of 2 or more. Following ORBS treatment, the average CAS was reduced to 0.38, with 65% of cases recording a CAS of 0 (clinically healthy), indicating a generalized shift among all treated animals toward a healthy clinical status. Within 48 h of ORBS treatment, all animals but one had a CAS value of 0 (mean CAS of 0.03).

**Table 3.** Mean blood gas values (means with SEM in parentheses) for healthy calves in a diarrhea and diarrhea-free environment

Blood gas variable	Healthy calves		Reference range <sup>1</sup>
	Diarrhea-free environment (farm A) (n = 28; 71 measurements)	Diarrhea environment (farms B, C, D) (n = 23; 25 measurements)	
pH	7.42 (0.004)	7.39 (0.006)*	7.31–7.53
HCO <sub>3</sub> <sup>-</sup> (mM)	29.78 (0.311)	30.19 (0.465)	17–29
Base excess (mM) <sup>2</sup> standard	6.00 (0.339)	7.09 (0.502)	0 ± 2
Anion gap (mM) <sup>2</sup>	12.77 (0.405)	10.27 (0.429)*	14–20
Na <sup>+</sup> (mM)	138.94 (0.317)	135.88 (0.617)*	132–152
K <sup>+</sup> (mM)	4.85 (0.040)	4.73 (0.096)	3.9–5.8
Cl <sup>-</sup> (mM)	99.56 (0.425)	96.64 (0.553)*	97–111
SID <sup>3</sup> (mM)	44.23 (0.411)	43.96 (0.457)	38–42
Glucose (mM)	7.91 (0.359)	5.71 (0.185)*	2.49–4.16
Ca <sup>2+</sup> (mM)	1.26 (0.007)	1.24 (0.011)	>1.0
Total hemoglobin (g/dL)	11.67 (0.183)	—	8.6–11.9

<sup>1</sup>Adult range for base excess was from Stämpfli et al. (2012); adult ranges for Ca<sup>2+</sup> and total hemoglobin were from Divers and Peek (2007); adult ranges for all other variables were from Smith (2014).

<sup>2</sup>Calculated using blood gas machine algorithm.

<sup>3</sup>Strong ion difference = [Na<sup>+</sup>] + [K<sup>+</sup>] - [Cl<sup>-</sup>].

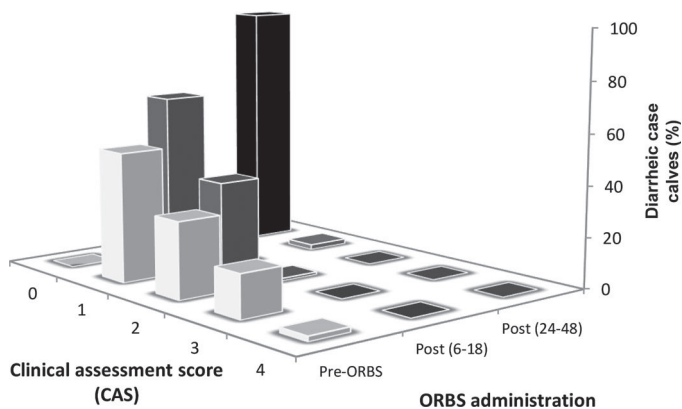
\*P = 0.001: statistical difference between pre- and posttreatment values estimated using linear regression.

The correlations between CAS and blood gas variables are presented in Table 4. The correlation estimates indicated that pH, HCO<sub>3</sub><sup>-</sup>, and BE were strongly and significantly correlated with CAS, with values exceeding 0.60 in all cases (P < 0.05). A further correlation analysis between HCO<sub>3</sub><sup>-</sup> concentration and SID yielded a correlation estimate of 0.78 (P < 0.0001). Graphical representations of 8 blood gas variables and CAS are presented in Figures 2 and 3. The final 3 variables are included in Supplementary Figure S2 (<http://dx.doi.org/10.3168/jds.2015-10600>).

Weight measurements recorded from research farm A in healthy and ORBS-treated diarrheic calves are pre-

sented in Figure 4; no significant difference in weights was identified at any time point (P > 0.05 in all cases). Before the outbreak, the diarrhea cohort had a (non-significant) heavier mean weight relative to the healthy cohort. In the 14-d period following the diarrhea outbreak, this weight advantage was temporarily reversed, indicating reduced growth rates in the diarrhea cohort. However, similar mean weights were recorded thereafter for both cohorts.

The assessment of the effect of sex, calving difficulty, breed, date of calving, weight, and single or twin on blood gas variables at birth indicated no significant associations.



**Figure 1.** Clinical assessment scores (CAS) for diarrheic calves, before and after treatment with an oral rehydration and buffering solution (ORBS). Pre-ORBS = before administration of ORBS; Post (6–18) = 6 to 18 h after administration of ORBS; Post (24–48) = 24 to 48 h after administration of ORBS.

**Table 4.** Spearman correlation coefficients (rho) between blood gas variables (reclassified as ordinal data) and calf clinical assessment score for all diarrheic study calves

Blood gas variable	Spearman rho
pH	-0.63*
HCO <sub>3</sub> <sup>-</sup>	-0.75*
Base excess standard	-0.74*
Anion gap	0.40*
Na <sup>+</sup>	-0.39*
K <sup>+</sup>	0.11
Cl <sup>-</sup>	-0.03
SID <sup>1</sup>	-0.59*
Glucose	-0.30*
Ca <sup>2+</sup>	-0.12
Total hemoglobin	0.23*

<sup>1</sup>Strong ion difference = [Na<sup>+</sup>] + [K<sup>+</sup>] - [Cl<sup>-</sup>].

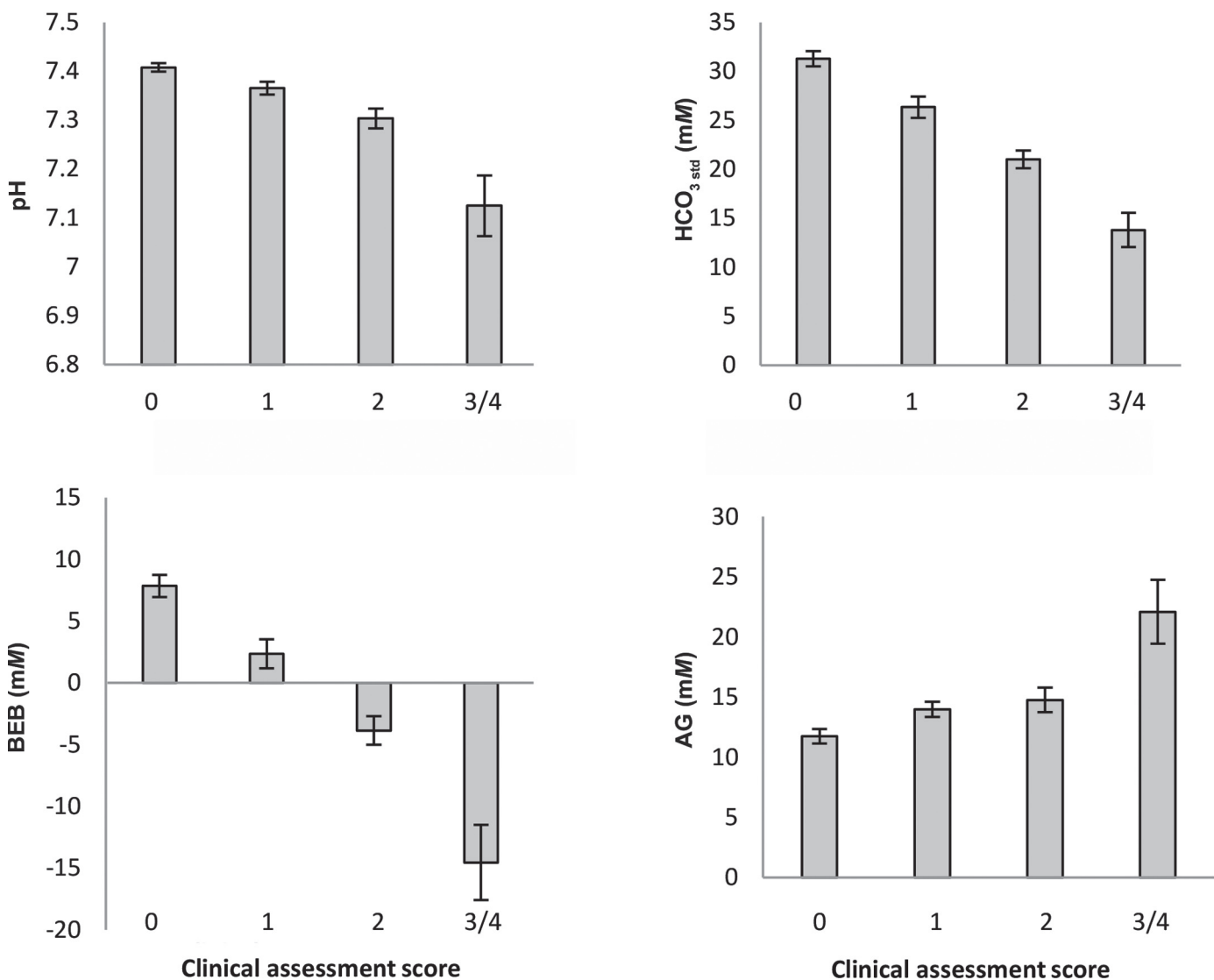
\*P < 0.05.



## DISCUSSION

Blood gas analysis was used in this observational study to assess both healthy and pre- and post-ORBS-treated diarrheic dairy calves. We found blood pH to be a simple and useful indicator of clinical health in study calves and it would be a useful diagnostic and prognostic tool at the farm level. Additionally, we observed that an ORBS that couples a high SID and a bicarbonate buffer, such as that used in this study, is an appropriate treatment for diarrheic calves. It effectively restored blood gas parameters to concentrations comparable to those of healthy animals, and all animals treated in the study recovered rapidly from diarrheic episodes.

The strong significant correlations we identified between CAS and pH,  $\text{HCO}_3^-$ , and BE, in particular, indicate that clinical health is determined more by bicarbonate concentration than by any of the electrolytes measured. This is in agreement with several previous studies (Kasari and Naylor, 1984; Naylor, 1989; Geishauser and Thünker, 1997; Wendel et al., 2001; Lorenz, 2004), where the link has been well established. Typically, a diarrheic calf will be hyponatremic and hypo- or hyperkalemic (Lewis and Phillips, 1973; Constable and Grünberg, 2013) based on the chronic or acute stage of the condition (Smith and Berchtold, 2014), respectively. However, the pretreatment diarrheic calves in this study had a wide range of electro-



**Figure 2.** Mean blood pH,  $\text{HCO}_3^-$ , base excess blood (BEB), and anion gap (AG) ( $\pm$ SEM) at each clinical assessment score (CAS); CAS groups 3 and 4 were merged for analysis. CAS 0: n = 18 (27 data points); CAS 1: n = 28 (27 data points); CAS 2: n = 10 (13 data points); CAS 3: n = 7 (18 data points), CAS 4: n = 1 (1 data point).

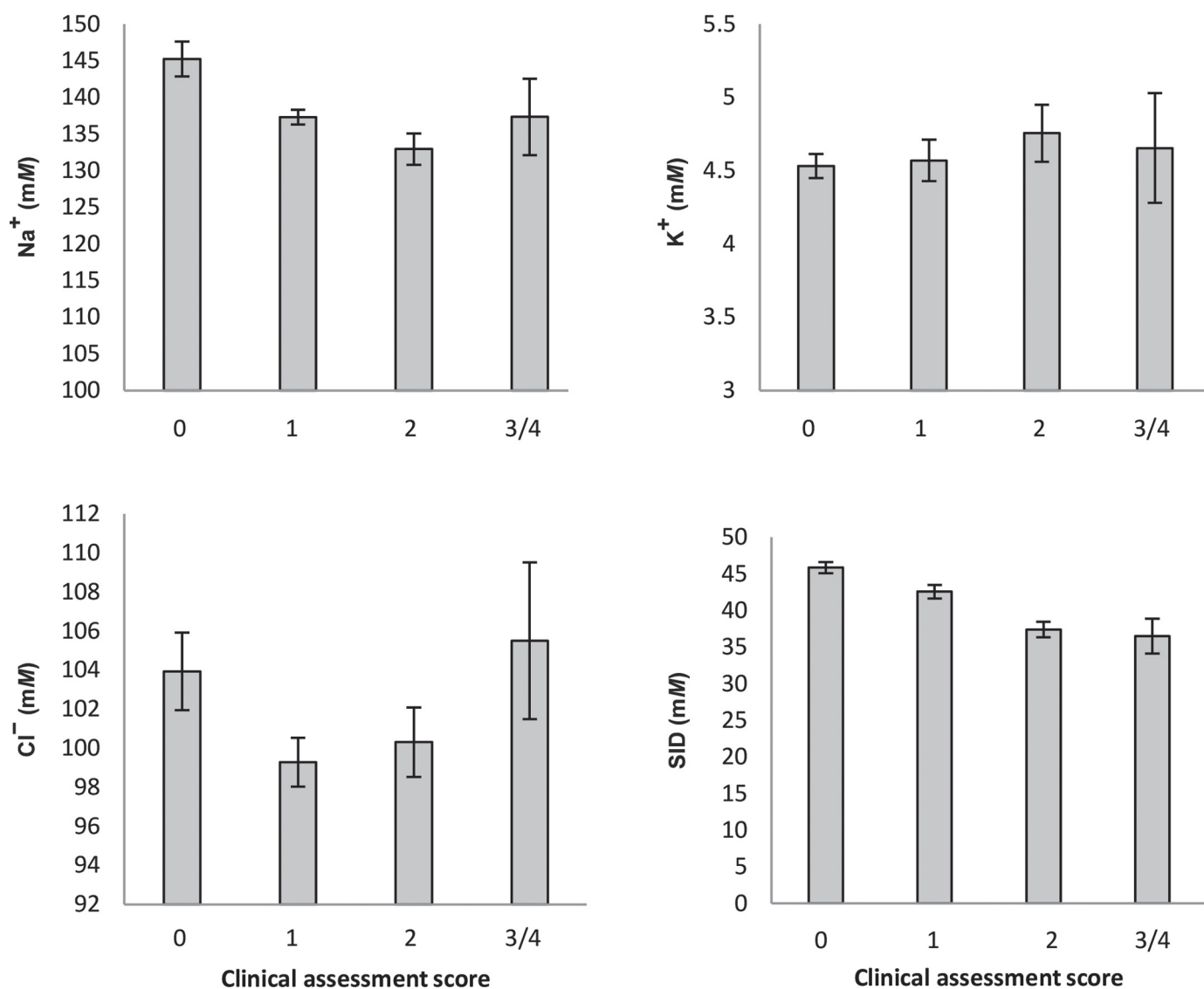
lyte concentrations, with no clear consensus as to a definitive hypo- or hyper- status for either sodium or potassium.

We suggest, therefore, that for an individual diarrheic calf, assessment of sodium and potassium electrolyte concentrations is an unreliable indicator of the severity of the diarrhea. However, the fact that bicarbonate was strongly associated with SID in these calves may support the theory that the relationship between these elements, in addition to chloride, is a more important associate to bicarbonate concentration than the individual elements themselves.

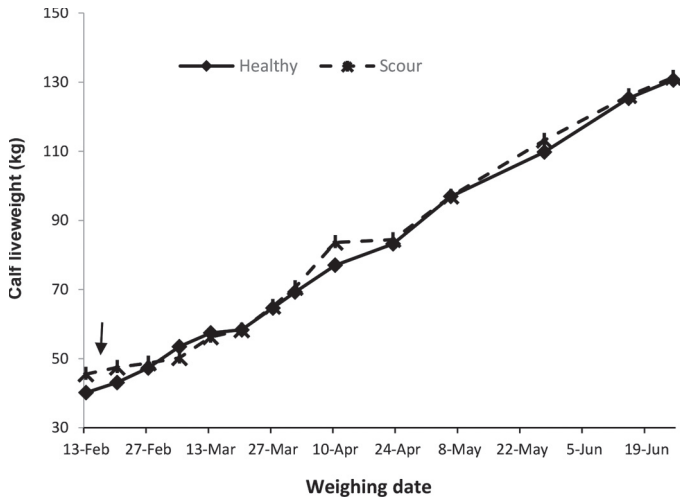
The CAS chart was used purely as a means of formalizing and standardizing assessment of calf health over

the duration of this study. It is not presented, nor is it intended, to act as a validated scoring tool to inform the timing of intervention or treatment of diarrheic calves. In this study, however, we have highlighted the parameters—blood pH, bicarbonate, BE, and AG—that should be used in validating such a scoring system.

The assessment of healthy calves, in both a diarrhea-free and a diarrheic environment, was broadly in line with previously published reference ranges (Divers and Peek, 2007; Smith, 2014). However, it should be noted that these reference values relate to adult bovine animals, because there is limited availability of and variable ranges (Slanina et al., 1992) for neonates in the literature. We identified possible exceptions to adult



**Figure 3.** Mean blood sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ) and strong ion difference (SID) ( $\pm$ SEM) at each clinical assessment score (CAS); CAS groups 3 and 4 were merged for analysis. CAS 0:  $n = 18$  (27 data points); CAS 1:  $n = 28$  (27 data points); CAS 2:  $n = 10$  (13 data points); CAS 3:  $n = 7$  (18 data points), CAS 4:  $n = 1$  (1 data point).



**Figure 4.** Weight measurement over time for healthy ( $n = 24$ ) and ORBS (oral rehydration and buffering solution)-treated diarrhea ( $n = 8$ ) calves. Data records were available for farm A calf cohort only. Arrow highlights commencement of diarrhea outbreak in this calf cohort.

reference ranges published previously. For example, the lower reference range pH value of 7.31, if theoretically applied to a calf in the current study, would be considered clinically unhealthy (CAS of 1). Reference ranges for bicarbonate, SID, and BE were also underestimated relative to the healthy animals in this study, and AG was overestimated. The timing of blood analysis relative to feeding is a possible factor to the variations in these variables. The animals in this study were measured approximately 2 h postfeeding. Age of the calf can also be a determining factor in acidemia (Naylor, 1989), with calves during their first week of life being less acidemic than older calves. Although we did not account for age in this study due to lack of accurate records on commercial study farms, the youngest diarrheic calf was 7 d old, and thus unlikely to be naturally less acidemic. Additionally, the fact that healthy calves reared in a diarrhea environment had significantly lower blood gas values, relative to those in a diarrhea-free environment, for 5 of the 11 variables investigated raises the possibility of management or environmental influences on blood gas parameters. As further data relating to blood gas measurements for healthy neonate calves emerge from future studies, taking feeding time, neonate age (Mohri et al., 2007), and environment stressors into account, it is likely that currently reported reference ranges need to be revised.

Blood gas analysis can be valuable for establishing baseline parameters, confirming a diagnosis, determining the prognosis, planning therapeutic options, and

monitoring response to treatment (Russell and Roussel, 2007), despite overestimating oxygen exchange fraction in some cases (Detry et al., 2003). The results of the current study support its usefulness in the field by allowing detection of electrolyte disturbance and acidosis in calves, and informative monitoring of calf recovery post-ORBS treatment. The high cost of blood gas analyzers and widespread use by veterinarians of clinical assessment alone in assessing calf diarrhea precludes the use of this accurate diagnostic tool at farm level. The strong correlation between pH and clinical health, as measured in this study, suggests that monitoring pH alone is useful, particularly as a means to monitor recovery following treatment. The availability of simplified, economical, and portable diagnostic equipment, such as a pocket blood pH meter, would therefore improve accurate diagnosis and prognosis based on our results. A suitable pH cut-off value below which (further) treatment is required would need to be established. Bleul et al. (2007) suggests a pH cut-off of 7.20 to classify newborn calves as acidotic, whereas the lower reference range for pH is 7.31 for older animals. However, based on this current analysis, a value closer to 7.36 (mean pH value for calves with a CAS of 1) may be more appropriate for calves aged 7 to 26 d. Further analysis on a larger data set would be of benefit in defining a suitable cut-off value.

We chose the ORBS used in the current study to reflect a new range of electrolyte treatments that meet the specifications of the modified EC directive. In Ireland, at least, all ORBS must conform to this directive. Although the exact formulation of the ORBS used in the current study was not disclosed by the manufacturer for commercial reasons, its use facilitated testing of a water-based ORBS differentiated by high SID. It included a bicarbonate buffer and additional ingredients, including fat-soluble vitamins. Although we cannot confirm that high SID alone is sufficient as the central component of an ORBS, it is evident that when it is combined with a buffering component, the objective of restoring calves to full health is achieved. The beneficial properties of sodium bicarbonate-based buffers in ORBS have been previously reported (Sen et al., 2009), and the results of the current study (reduction in CAS and normalization of blood gas parameters) suggest that coupling a high-SID ORBS with a buffering component yields an effective diarrhea treatment. However, the ORBS we used contains additional supplements, such as tocopherol. Its contribution to the efficacy of the product cannot be disregarded in light of the important role that fat-soluble vitamins play in maintaining calf health (Torsein et al., 2011).



## CONCLUSIONS

Administration of an ORBS formulated on a principle of high SID coupled with a bicarbonate buffer and supplementary nutritional ingredients resulted in rapid recovery from a diarrheic episode in dairy calves. Additionally, we observed measurement of blood pH to be a useful and practical tool for monitoring calf recovery following treatment for diarrhea.

## ACKNOWLEDGMENTS

We acknowledge financial support from Epsilon Ltd. (Cork, Ireland) through the Enterprise Ireland Innovation Voucher scheme (IV20151256), which supported 30% of the research costs and supplied product without charge. We sincerely thank all the farm staff at Moorepark (Co. Cork, Ireland) and on commercial farms for access to and care of calves included in the study.

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