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Use of a Quantitative Strong Ion Approach to Determine the Mechanism for Acid–Base Abnormalities in Sick Calves with or without Diarrhea

Peter D. Constable, Henry R. Stämpfli, Hérve Navetat, Joachim Berchtold, and François Schelcher

Acid-base abnormalities are frequently present in sick calves. The mechanism for an acid-base disturbance can be characterized using the strong ion approach, which requires accurate values for the total concentration of plasma nonvolatile buffers (A_{tot}) and the effective dissociation constant for plasma weak acids (K_a). The aims of this study were to experimentally determine A_{tot}, K_a, and net protein charge values for calf plasma and to apply these values quantitatively to data from sick calves to determine underlying mechanisms for the observed acid-base disturbance. Plasma was harvested from 9 healthy Holstein-Friesian calves and concentrations of quantitatively important strong ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, L-lactate) and nonvolatile buffer ions (total protein, albumin, phosphate) were determined. Plasma was tonometered with CO_2 at 37°C, and plasma P_{CO_2} and pH measured over a range of 15–159 mm Hg and 6.93–7.79, respectively. Strong ion difference (SID) was calculated from the measured strong ion concentrations, and nonlinear regression was used to estimate values for A_{tot} and K_a from the measured pH and P_{CO_2} and calculated SID. The estimated A_{tot} and K_a values were then validated using data from 2 in vivo studies. Mean (± SD) values for calf plasma were A_{tot} = 0.343 mmol/g of total protein or 0.622 mmol/g of albumin; $K_a = (0.84 \pm 0.41) \times 10^{-7}$; $pK_a = 7.08$. The net protein charge of calf plasma was 10.5 mEq/L, equivalent to 0.19 mEq/g of total protein or 0.34 mEq/g of albumin. Application of the strong ion approach to acid-base disturbances in 231 sick calves with or without diarrhea indicated that acidemia was due predominantly to a strong ion acidosis in response to hyponatremia accompanied by normochloremia or hyperchloremia and the presence of unidentified strong anions. These results confirm current recommendations that treatment of acidemia in sick calves with or without diarrhea should focus on intravenous or PO administration of a fluid containing sodium and a high effective SID.

Key words: Calf diarrhea; D-lactic acidosis; Hyponatremia; Metabolic acidosis.

Acidemia and metabolic acidosis occur commonly in sick calves with or without diarrhea. Metabolic acidosis in diarrheic calves was originally attributed to fecal bonate loss as well as the presence of unidentified organic acids in plasma and a decrease in glomerular filtration rate in response to severe dehydration.[1–4] These proposed mechanisms for development of metabolic acidosis were based, in part, on the presence of hyper-L-lactatemia in diarrheic calves,[1,2,5,6] hyper-L-lactatemia and extensive loss of bicarbonate in the watery stool of humans with cholera,[7] and from (personal communication) of a high fecal bicarbonate concentration (40 mEq/L) in 4 calves with experimentally induced enterotoxigenic *Escherichia coli* diarrhea.[c] However, metabolic acidosis in diarrheic calves is probably predominantly due to causes other than fecal bicarbonate loss because diarrheic calves have measured mean daily fecal losses of sodium (10.3 mEq/kg body weight), potassium (1.7 mEq/kg body weight), and chloride (7.9 mEq/kg body weight), producing an estimated daily fecal unmeasured anion loss of 4.1 mEq/kg body weight.[8] The estimated daily fecal unmeasured anion loss is equivalent to a maximal reduction in plasma bicarbonate concentration of 8.2 mEq/L for each day of diarrhea, assuming that the distribution space for bicarbonate approximates the extracellular fluid volume of calves (50% of body weight), and that the concentration of unmeasured fecal anions (such as acetate and D-lactate) is negligible. The latter assumption is clearly incorrect, in that median concentrations of acetate and D-lactate in the feces of diarrheic calves are 23 and 25 mmol/L, respectively.[9] In other words, reduction in plasma bicarbonate concentration due to fecal bicarbonate loss is likely to be substantially less than 8.2 mEq/L for each day of diarrhea. Moreover, net bicarbonate movement across the calf's intestine is similar in healthy and diarrheic calves.[10] Taken together, these results indicate that fecal bicarbonate loss is probably not the major cause of metabolic acidosis in diarrheic calves.

Despite the findings of early studies in diarrheic calves that identified hyper-L-lactatemia (mean blood or plasma lactate concentration = 5.1,[1] 3.6,[4,6] or 2.2[2] mmol/L; reference range, 0.6–1.9 mmol/L[1]) and that blood L-lactate concentration was related to the severity of dehydration in calves <1 week of age,[6] metabolic acidosis in diarrheic calves is not predominantly due to hyper-L-lactatemia in response to dehydration and poor tissue perfusion. Recent studies have indicated that serum L-lactate concentrations were similar in diarrheic and healthy calves[9] and that severe dehydration produces only a mild L-lactic acidosis in calves.[11] Furthermore, D-lactic acidosis has been identified as an important factor in the development of acidemia in diarrheic calves.[9] It therefore appears that metabolic acidosis in diarrheic calves probably arises from factors other than fecal bicarbonate loss and hyper-L-lactatemia as a result of dehydration and decreased tissue perfusion.

From the Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL (Constable); Department of Clinical Studies, University of Guelph, Guelph Ontario, Canada (Stämpfli); Center for Clinical Veterinary Research, Le Donon, France (Navetet); Veterinary Practice, Obing, Germany (Berchtold); l'Ecole Nationale Vétérinaire de Toulouse, Toulouse, France (Schelcher). *Material contained in this paper was presented at the VIth Congress of the International Society of Animal Clinical Biochemistry, Guelph, Canada, August 1994*[a] *and the 23rd World Buiatrics Congress in Quebec City, Quebec, Canada, July 2004.*[b]

Reprint requests: Peter D. Constable, PhD, Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, 1008 W. Hazelwood Drive, Urbana, IL 61802; e-mail: constable@uiuc.edu.

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Since the first report of hyper-D-lactatemia in sick calves in 1997,[12] it has become clear that metabolic acidosis in calves can result from an increase in plasma strong anion concentrations such as D-lactate after ruminal or large intestinal fermentation of milk and oral electrolyte solutions.[9,13–16] A decrease in plasma strong ion difference (SID; the difference in concentration between plasma strong cations and anions) directly causes a strong ion acidosis and acidemia.[17,18] Plasma SID is decreased by hyper-D-lactatemia, hyper-L-lactatemia, and hyponatremia accompanied by normochloremia or hyperchloremia; these changes are usually present in diarrheic calves.[1,3,19,20] Dehydration increases the plasma protein concentration, and such an increase causes a nonvolatile buffer ion acidosis and acidemia.[17,18] The relative contribution of hyper-D-lactatemia, hyper-L-lactatemia, and hyponatremia, accompanied by normochloremia or hyperchloremia, and increased plasma protein concentration to the severity of the acidemia in sick calves with and without diarrhea is currently unknown, although hyper-D-lactatemia appears to be quantitatively more important than hyper-L-lactatemia.[9,15,16]

Acid-base balance has traditionally been evaluated by using the Henderson-Hasselbalch equation to characterize 4 primary acid-base disturbances (ie, respiratory acidosis and alkalosis, metabolic acidosis, and alkalosis)[21] and by calculating the anion gap to determine whether metabolic acidosis is due to the loss of bicarbonate or the increase in unmeasured plasma anion concentrations such as L-lactate or D-lactate.[21–24] However, because the anion gap is altered by changes in serum protein concentration and therefore the net anionic charge on serum proteins, the anion gap does not accurately quantify the unmeasured strong ion charge in calves with an abnormal serum protein concentration as a result of dehydration or inadequate absorption of colostral immunoglobulins. The first objective was therefore to calculate the net protein charge in calf plasma in order to more accurately quantify the unmeasured strong ion charge in sick calves with or without diarrhea.

Two mechanistic physicochemical approaches have been developed to evaluate acid-base balance; Stewart's strong ion model[17] and the simplified strong ion model.[18] The strong ion approach states that 3 independent variables (plasma SID, P_{CO_2}, and plasma nonvolatile buffer ion concentration) directly determine plasma pH and that 6 primary acid-base disturbances (ie, respiratory acidosis and alkalosis, strong ion acidosis and alkalosis, nonvolatile buffer ion acidosis and alkalosis) exist.[21] Because an accurate understanding of the mechanism for an acid-base abnormality may lead to better treatment protocols, the second objective was to use the strong ion approach to characterize the mechanism for acid-base abnormalities in diarrheic and sick calves.

The strong ion approach requires species-specific values for the total plasma concentration of nonvolatile weak acids (A_{tot}; ie, the total concentration of plasma nonvolatile buffers; albumin, globulin, and phosphate) and the effective dissociation constant (K_a) for plasma nonvolatile buffers.[18,25–28] Values for A_{tot} and K_a have been experimentally determined in the plasma of horses,[18,25] humans,[26] cats,[27] and dogs,[d] and theoretically determined for the plasma of cattle.[28] We hypothesized that A_{tot} and K_a values for plasma of calves were similar to those of adult cattle, but different from those of other species. Accordingly, the third objective of the study reported here was to experimentally determine A_{tot} and K_a values for the plasma of calves and to compare these values with A_{tot} and K_a values for the plasma or adult cattle, horses, cats, dogs, and humans.

Materials and Methods

Blood and Plasma Collection

Twenty mL of venous blood was collected into lithium-heparin tubes from the jugular vein of 4 female and 5 male healthy Holstein-Friesian calves, 4–55 days of age. Plasma was harvested by centrifugation within 30 minutes of collection and stored for up to 2 months at −70°C. Plasma was thawed at room temperature immediately before CO_2 tonometry was performed.

CO_2 Tonometry of Plasma

Plasma samples were tonometered[e] for 20 minutes at 37°C over a P_{CO_2} range of 15–159 mm Hg and a pH range of 7.79–6.93 using a mixture of humidified 20% CO_2 and 100% O_2. A total of 117 CO_2 tonometered plasma samples were analyzed, and these samples represented 8–16 different P_{CO_2} values for each calf.

Blood and Plasma Analyses

The jugular venous blood sample and all tonometered plasma samples were analyzed[f] in duplicate for blood/plasma gas analysis (pH, P_{CO_2}) and determination of [Na^+], [K^+], [Ca^{2+}], [Cl^-], and [L-lactate$^-$]. An untonometered plasma sample was analyzed[g] in duplicate for determination of strong cation (Mg^{2+}) and nonvolatile buffer ion (total protein, albumin, and inorganic phosphate) concentrations.

Calculation of SID

All strong cation (Na^+, K^+, Ca^{2+}, Mg^{2+}) and strong anion (Cl^-, L-lactate) concentrations were assumed to be constant during CO_2 tonometry. Accurate measurements of SID are difficult to obtain in plasma due to cumulative measurement error, presence of unknown strong anions, and differences in equipment and methodology used to measure strong ion concentrations.[18,21,26,27] Accordingly, SID was initially calculated using 3 methods: $SID_3 = [([Na^+] + [K^+]) - ([Cl^-])]$; $SID_4 = [([Na^+] + [K^+]) - ([Cl^-] + [L\text{-lactate}])]$; $SID_6 = [([Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}]) - ([Cl^-] + [L\text{-lactate}])]$, and a fixed value for SID_3, SID_4, and SID_6 assigned using the mean value for all CO_2 tonometered samples from each calf. A fixed value for SID during in vitro CO_2 tonometry is one of the assumptions of the strong ion approach; SID is invariant over the physiologic range of pH because strong ions are fully dissociated at physiologic pH.[17,18]

Estimation of A_{tot} and K_a

Measured values for pH and P_{CO_2}, calculated values for SID_3, SID_4, and SID_6, the 6-factor simplified strong ion electroneutrality equation,[18] and the Marquardt nonlinear regression procedure[29,h] were used to simultaneously estimate values for A_{tot} and K_a. This required application of the 6-factor simplified strong ion electroneutrality equation,

$$SID - [HCO_3^-] - [A^-] = 0, \quad (1)$$

in which the net nonvolatile buffer ion concentration in plasma ([A^-]) was evaluated. The 6-factor simplified strong ion model[18] was used instead of Stewart's 8-factor strong ion model[17] because reducing the number of variables in a nonlinear regression model leads to more precise estimates[29] and because changes in 2 of the 8 factors in Stewart's electroneutrality equation do not produce changes in pH and are therefore redundant.[18,28]

To assist in estimating values for A_{tot} and K_a, Equation (1) was expressed in the following form, of which the left 2 parts are a restatement of the traditional Henderson-Hasselbalch equation:

$$[HCO_3^-] = S \cdot PCO_2 \cdot 10^{(pH - pK1')} = SID - (A_{tot} \cdot K_a)/(K_a + 10^{-pH}) \quad (2)$$

using known values for S (0.0307 [mmol/L]/mm Hg)[30] and pK_1' (6.120 at [NaCl] = 0.16 mmol/L).[31] Using the value of 6.120 for pK_1' calculates actual plasma $[HCO_3^-]$ in (mmol/L) at 37°C[31]; likewise, the methods used to calculate SID provide a value in terms of concentration. This means that Equation (2) estimates a value for A_{tot} in terms of concentration (mmol/L). The form of the simplified strong ion electroneutrality equation used in Equation (2) was selected because it provided the narrowest confidence intervals for the estimated values of A_{tot} and K_a in this study. Initial estimates for A_{tot} of 5–30 mmol/L in increments of 5 mmol/L and initial estimates for K_a of 0.1×10^{-7} to 3.0×10^{-7} in increments of 0.1×10^{-7} were used for the nonlinear regression procedure. For each nonlinear regression procedure, the accuracy of the estimated values for A_{tot} and K_a were evaluated using the number of iterations required to converge to a solution, calculating the R^2 value, comparison of actual versus predicted values for $[HCO_3]$, and examination of residual plots. A value of $P < .05$ was regarded as significant.

The true value for SID was unknown. Therefore, a fourth nonlinear regression procedure was performed to simultaneously estimate values for A_{tot}, K_a, and SID (called $SID_{estimated}$), with initial estimates for $SID_{estimated}$ of 30–50 mEq/L in increments of 5 mEq/L. R^2 values were calculated for the 4 nonlinear regression models (on the basis of SID_3, SID_4, SID_6, $SID_{estimated}$), using the values obtained during CO_2 tonometry of each plasma sample. The calculated A_{tot} values were evaluated as the A_{tot} indexed to the total protein concentration ($A_{tot\text{-}tp}$) and the A_{tot} indexed to the albumin concentration ($A_{tot\text{-}alb}$). Mean (± SD) values for A_{tot}, $A_{tot\text{-}tp}$, $A_{tot\text{-}alb}$, and K_a were determined.

Validation of A_{tot} *and* K_a *Values*

The estimated A_{tot} and K_a values were applied to data obtained from in vivo studies involving 8 healthy dairy calves with acute mixed respiratory and metabolic acidosis induced by halothane anesthesia and intravenous administration of L-lactic acid,[i] and 5 healthy dairy calves suckling a bicarbonate containing oral electrolyte solution (unpublished data). Plasma pH was calculated using the simplified strong ion equation,[18]

$$pH = \log_{10}\left(2 \cdot SID \Big/ \left\{K_1 \cdot S \cdot PCO_2 + K_a \cdot A_{tot} - K_a \cdot SID + \sqrt{\{(K_1 \cdot S \cdot PCO_2 + K_a \cdot SID + K_a \cdot A_{tot})^2 - 4 \cdot K_a^2 \cdot SID \cdot A_{tot}\}}\right\}\right) \quad (3)$$

from calculated values for SID and reported values for PCO_2 and total protein or albumin concentration; SID was calculated as SID = $[Na^+] + [K^+] - [Cl^-] -$ [L-lactate], where $[Na^+]$, $[K^+]$, and $[Cl^-]$ were determined by ion-selective electrode potentiometry[j,k] and [L-lactate] was determined by the L-lactate dehydrogenase method.[l] A_{tot} was calculated from the total protein concentration, which was measured by the biuret method.[i] The calculated pH value (pH_{calc}) was regressed against the measured pH value (pH_{meas}) using a statistical software package,[m] and a value of $P < .05$ was regarded as significant. The accuracy of the 4 linear regression equations was evaluated using the R^2 value and examination of residual plots. A value of $P < .05$ was regarded as significant.

Comparison of A_{tot} *and* K_a *Values*

The estimated values for A_{tot} and K_a in plasma of calves were compared with those obtained for plasma of horses,[18,25] humans,[26] cats,[27] and dogs[d] by using an unpaired *t*-test.[n] Because 5 multiple comparisons were being performed, the P value for significance was Bonferonni adjusted for the number of comparisons, producing a $P < .01$ as significant.

Determining the Mechanism for Acid-Base Disturbances in Sick Calves

The major obstacle to the clinical application of the strong ion approach is obtaining an accurate value for SID.[18,21–28] Determination of SID requires identification and accurate measurement of all strong ions in plasma, which is an impossible task because high concentrations of unidentified strong anions, such as L-lactate, D-lactate, and uremic anions, may be present in sick calves[23] and due to cumulative measurement error. However, because SID has 2 components, measured strong ion difference (SID_m) and unmeasured strong ion difference (SID_{um}),[24] the following equation is true:

$$SID = SID_m + SID_{um} \quad (4)$$

Rearrangement of Equation (2) and substitution for SID using Equation (4) produces the following equation:

$$SID_{um} = S \cdot PCO_2 \cdot 10^{(pH - pK1')} + \{A_{tot}/[1 + 10^{(pKa - pH)}]\} - SID_m \quad (5)$$

It is important to consider both SID_m and SID_{um} when calculating SID because utilizing only SID_m (as $SID_m = ([Na^+] + [K^+] - [Cl^-])$ in calves with increased concentrations of other strong anions (such as L-lactate, D-lactate) does not provide a useful insight into the mechanism for an acid-base disturbance in diarrheic calves.[32]

The strong ion approach indicates that we can determine the mechanism for an acid-base disturbance in calves by regressing the dependent variable (hydrogen ion activity = a_{H+}; pH = $-\log_{10} a_{H+}$) against the 3 independent variables (SID, PCO_2, A_{tot}):

$$a_{H+} = \left\{K_1 \cdot S \cdot PCO_2 + K_a \cdot A_{tot} - K_a \cdot SID + \sqrt{\{(K_1 \cdot S \cdot PCO_2 + K_a \cdot SID + K_a \cdot A_{tot})^2 - 4 \cdot K_a^2 \cdot SID \cdot A_{tot}\}}\right\} \div (2 \cdot SID) \quad (6)$$

This form of the simplified strong ion equation was selected for regression analysis because it describes a more linear relationship among a_{H+} and SID, PCO_2, A_{tot} than does Equation (3) and therefore is more appropriate for stepwise linear regression analysis. Accordingly, values for a_{H+}, measured strong ion concentrations (SID_m), PCO_2, and total protein concentration were extracted from 4 different data sets of calves with naturally acquired or experimentally induced diarrhea, calves with chronic indigestion due to ruminal drinking, and calves with septicemia and diarrhea.

The first data set (study 1) consisted of the results of jugular venous blood analysis from 81 sick Charolais calves with diarrhea but variable degrees of dehydration (0–9% bodyweight, on the basis of extent of eye recession into the orbit). The second data set (study 2) consisted of the results of jugular venous blood analysis from 64 Charolais calves with experimentally induced enterotoxigenic *Escherichia coli* diarrhea. The third data set (study 3) consisted of the published results of jugular venous blood analysis from 42 Brown Swiss, 1 Holstein, and 7 Simmental calves aged 6 days to 3 months with general malaise, poor appetite, and metabolic acidosis as a consequence of ruminal drinking syndrome.[33] The fourth data set (study 4) consisted of the results of jugular venous blood analysis from 27 Holstein, 2 German-Angus, 1 German Simmental, and 6 Holstein-crossbreed calves aged 3–28 days with metabolic acidosis and diarrhea or respiratory disease.[34] To assist in comparing the results of analysis, the value for SID_m was calculated from the measured serum sodium, potassium, and chloride concentrations ($SID_m = [Na^+] + [K^+] - [Cl^-]$), and the value for A_{tot} was calculated from the measured serum total protein concentration or plasma protein concentration.

Forward stepwise regression ($P < .05$ to enter and exit) was performed separately on the 4 data sets to determine the relationship among a_{H+} and SID_m, SID_{um} (calculated using Equation (5)), PCO_2, and A_{tot}. The order of entry of variables into the regression model and partial R^2 values were used to determine the relative importance and contribution of each variable to the acid-base derangement.[29] Subsequently, stepwise regression was used to determine the relative con-

Table 1. Summary of mean (± SD) estimated values for SID, K_a, A_{tot}, $A_{tot\text{-}alb}$, and $A_{tot\text{-}tp}$ for 9 healthy calves.

Measurement Method	SID (mEq/L)	K_a ($\times 10^{-7}$)	A_{tot} (mmol/L)	$A_{tot\text{-}alb}$ (mmol/g)	$A_{tot\text{-}tp}$ (mmol/g)
SID_3	43.0 ± 2.4	1.08 ± 0.49	20.1 ± 4.7	0.654 ± 0.150	0.359 ± 0.102
SID_4	41.1 ± 2.7	0.84 ± 0.41	19.2 ± 6.1	0.622 ± 0.191	0.343 ± 0.127
SID_6	45.4 ± 3.6	1.68 ± 0.64	20.8 ± 3.9	0.676 ± 0.117	0.370 ± 0.084
$SID_{estimated}$	44.4 ± 3.6	0.85 ± 0.32	23.1 ± 6.1	0.750 ± 0.187	0.411 ± 0.122

SID, strong ion difference, which is the difference in charge between plasma strong cations and anions; K_a, effective dissociation constant for plasma nonvolatile weak acids; A_{tot}, total plasma concentration of nonvolatile weak acids; $A_{tot\text{-}alb}$, A_{tot} indexed to the plasma albumin concentration; $A_{tot\text{-}tp}$, A_{tot} indexed to the plasma total protein concentration; SID_3, ([Na^+] + [K^+]) − [Cl^-]; SID_4, ([Na^+] + [K^+]) − ([$C1^-$] + [L-lactate]); SID_6, ([Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}]) − ([Cl^-] + [L-lactate]); $SID_{estimated}$, a nonlinear regression procedure was used to simultaneously estimate values for A_{tot}, K_a, and SID.

tribution of [Na^+], [K^+], [Cl^-], [L-lactate], and [D-lactate] to a_{H+}. The accuracy of the forward stepwise regression procedure was evaluated by examining the residual plots and confirming that a regression procedure using backward elimination ($P > .05$ to exit) produced the same final regression equation. A value of $P < .05$ was regarded as significant.

Sensitivity of Plasma pH to Changes in SID, P_{CO_2}, and A_{tot}

Sensitivity of the dependent variable (plasma pH) to the 3 independent factors (SID, P_{CO_2}, and A_{tot}) of the simplified strong ion model were graphically depicted by a spider plot,[35] which conveys the relationship between the dependent variable (pH) and percentage change in 1 of the 3 independent factors while the remaining 2 independent factors were held constant. The spider plot was created using Equation (3) and typical values for the plasma of healthy Holstein bull calves (n = 18), 3–14 days of age (pH = 7.38 ± 0.03; SID = 42.0 ± 2.4 mEq/L; P_{CO_2} = 53 ± 4 mm Hg; total protein concentration = 54.1 ± 0.4 g/L; unpublished data), as well as known values for S = 0.0307 [mmol/L]/mm Hg,[30] pK_1' = 6.120,[31] and estimated values for A_{tot}, and pK_a. The mean SID value of 42 mEq/L reflects the true mean SID for the plasma of healthy calves; this value was calculated for each calf from other measured variables using an algebraic rearrangement of Equation (2). Individual data points from the 4 studies involving diarrheic or sick calves were superimposed on the spider plot using reported values for pH, P_{CO_2}, total protein concentration, and SID_m ([Na^+] + [K^+] − [Cl^-]), known values for S = 0.0307 [mmol/L]/mm Hg[30] and pK_1' = 6.120,[31] and estimated values for A_{tot} (indexed to total protein concentration) and pK_a, and a calculated value for SID_{um} using Equation (5). SID was calculated from SID_m and SID_{um} as in Equation (4).

Derivatives of pH with respect to the 3 independent factors (SID, P_{CO_2}, A_{tot}) were then calculated as described[36] to provide an index of the sensitivity of plasma pH to changes in each of the independent factors at a reference pH of 7.38.

Results

Calculation of SID

Small differences in the mean value for SID_3 (43.0 mEq/L), SID_4 (41.1 mEq/L), SID_6 (45.4 mEq/L), and $SID_{estimated}$ (44.4 mEq/L) were obtained (Table 1).

Estimation of A_{tot} and K_a

The R^2 value for all nonlinear regression models obtained using Equation (2) were >.98, indicating excellent fit to the data. The estimated values for A_{tot} and K_a were similar, with slight variations occurring dependent on the value assigned to SID (Table 1).

Validation of A_{tot} and K_a Values

The estimated A_{tot} and K_a values were applied to data obtained from an in vivo study involving 8 calves with acute mixed respiratory and metabolic acidosis and 5 calves suckling a bicarbonate-containing oral electrolyte solution. All 4 pairs of A_{tot} and K_a values (from Table 1) were not statistically different from the line of identity (slope = 1, intercept = 0) relating calculated pH to measured pH, with $R^2 > .90$ for all regression analyses (data not shown). However, on the basis of the R^2 values, the best fitting values were derived from SID_4; A_{tot} = (19.2 ± 6.1) mmol/L (equivalent to 0.343 mmol/g of total protein or 0.622 mmol/g of albumin); $K_a = (0.84 \pm 0.41) \times 10^{-7}$; pK_a = 7.08.

Comparison of A_{tot} and K_a Values

The A_{tot} values of calf plasma were similar to those for dog, cat, and human plasma, but different ($P < .001$) from that for horse plasma. The K_a values of calf plasma were similar to those for cat and human plasma, but different ($P < .001$) from those for dog and horse plasma.

Determining the Mechanism for Acid-Base Disturbances in Sick Calves

Application of the strong ion approach and forward stepwise regression using jugular venous a_{H+} as the dependent variable and jugular venous SID_m, SID_{um}, P_{CO_2}, and A_{tot} values as independent variables from calves in studies 1, 2, 3, and 4 indicated that acidemia was due primarily to strong ion acidosis, with SID accounting for 58% to 75% of the change in pH (Fig. 1; Table 2). The strong ion acidosis was due to a decrease in SID_m (R^2 ranging from 26% to 62%) and SID_{um} (R^2 ranging from 8% to 49%), indicating that an increased unmeasured strong anion concentration was an important contribution to the acidemia. Although changes in P_{CO_2} accounted for 8–25% of the change in jugular venous a_{H+}, this change was a partially compensating respiratory alkalosis (Fig. 2). Acidemia was also due to a nonvolatile buffer ion acidosis, with A_{tot} accounting for 3% to 9% of the change in jugular venous a_{H+} (Fig. 3).

The relative contribution of changes in the measured strong cation ([Na^+] + [K^+]) and anion ([Cl^-]) concentra-

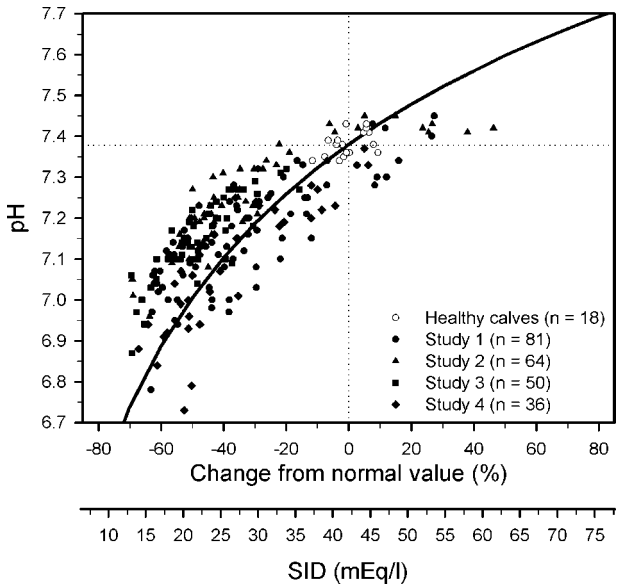

Fig. 1. Spider plot revealing the dependence of plasma pH on changes in the plasma strong ion difference (SID; solid line). The spider plot was obtained by systematically varying SID while holding the other 2 input variables (P_{CO_2} and A_{tot}, total concentration of nonvolatile weak acids) at their reference values for the plasma of healthy calves (P_{CO_2}, 53 mm Hg; A_{tot}, 18.5 mmol/L). The dotted lines indicate that pH = 7.38 when SID is at its reference value for the plasma of healthy calves (42 mEq/L). Individual data points for pH and SID obtained from 4 studies involving 231 sick calves with and without diarrhea (filled symbols) are clustered around the pH-SID relationship, thereby indicating that acidemia (pH < 7.38) was associated with a strong ion acidosis.

tions to change in a_{H+} were determined using forward stepwise regression to regress a_{H+} against [Na^+], [K^+], and [Cl^-]. The partial R^2 values for significant ($P < .05$) variables in the regression model were: study 1 ([Cl^-], partial $R^2 = .21$, $P < .0001$; [Na^+], partial $R^2 = .28$, $P < .0001$); study 2 ([Na^+], partial $R^2 = .22$, $P < .0001$; [Cl^-], partial $R^2 = .39$, $P < .0001$); study 3 (no variables were significant at $P < .05$); study 4 ([Cl^-], partial $R^2 = .22$, $P = .0067$). In the second study, where [D-lactate] was measured, the relationship between change in [D-lactate] and change in a_{H+} was determined by regressing a_{H+} against [D-lactate]. The R^2 value was .26 ($P < .0001$), indicating that an increase in plasma D-lactate concentration was statistically related to jugular venous a_{H+}. In the fourth study, where [L-lactate] was measured, [L-lactate] was not statistically related ($P = .41$) to a_{H+}.

Sensitivity of Plasma pH to Changes in SID, P_{CO_2}, and A_{tot}

Analysis of the spider plots revealed that plasma pH was most sensitive to changes in SID (Figs. 1– 3). The tangent to each line in the spider plot reflects the sensitivity of calf plasma pH to that factor, whereby, at the reference value for pH in samples obtained from the jugular vein (ie, pH 7.38), $d\text{pH}/d\text{SID} = +0.013$ (mEq/L)$^{-1}$, $d\text{pH}/d\text{P}_{CO_2} = -0.007$ mm Hg^{-1}, $d\text{pH}/A_{tot} = -0.003$ (mmol/L)$^{-1}$ = -0.001 (g total protein/L)$^{-1}$. This indicated that, at pH of 7.38, a 1-mEq/L decrease in SID will decrease pH by 0.013, a 1-mm Hg decrease in P_{CO_2} will increase pH by 0.007, and a 1-mmol/L decrease in A_{tot} will increase pH by 0.003.

Table 2. Determining the mechanism for acid-base disturbances in sick calves with or without diarrhea. The results of forward stepwise regression of jugular venous hydrogen ion activity (a_{H+}) as the dependent variable versus jugular venous SID_m (measured strong ion difference), SID_{um} (unmeasured strong ion difference), P_{CO_2}, and A_{tot} (total concentration of nonvolatile weak acids) are presented. Values were obtained from 81 diarrheic calves with variable dehydration (0–9% bodyweight; study 1), 64 calves with experimentally induced enterotoxigenic *Escherichia coli* diarrhea (study 2), 50 sick calves with chronic indigestion (study 3), and 36 calves with naturally acquired diarrhea and septicemia (study 4). The order of entry of variables into the regression model and partial R^2 values were used to determine the relative importance and contribution of SID_m, SID_{um}, P_{CO_2}, and A_{tot} to the acid-base derangement. The P value was <0.0001 for the coefficients of all variables.

Order of Entry into Regression Model	Variable	Partial R^2	Model R^2
Study 1			
1	SID_m	0.413	0.413
2	SID_{um}	0.162	0.575
3	P_{CO_2}	0.247	0.822
4	A_{tot}	0.050	0.872
Study 2			
1	SID_m	0.622	0.622
2	SID_{um}	0.099	0.721
3	P_{CO_2}	0.098	0.819
4	A_{tot}	0.034	0.853
Study 3			
1	P_{CO_2}	0.231	0.231
2	SID_m	0.080	0.311
3	SID_{um}	0.499	0.810
4	A_{tot}	0.093	0.903
Study 4			
1	SID_m	0.263	0.263
2	SID_{um}	0.490	0.753
3	P_{CO_2}	0.079	0.833
4	A_{tot}	0.067	0.900

Net Protein Charge

Net protein charge can have 2 components: strong ion charge and nonvolatile buffer ion charge. Because the mean value for SID of calf plasma was calculated to be 42.0 mEq/L (see earlier), the estimated strong ion charge of protein in calf plasma approximates zero because 42 mEq/L is within the 95% confidence intervals for SID_3, SID_4, SID_6, and $SID_{estimated}$ (Table 1).

The value for nonvolatile buffer ion charge ([A^-]) at pH = 7.38 (normal plasma pH for calves) reflects the pH-de-

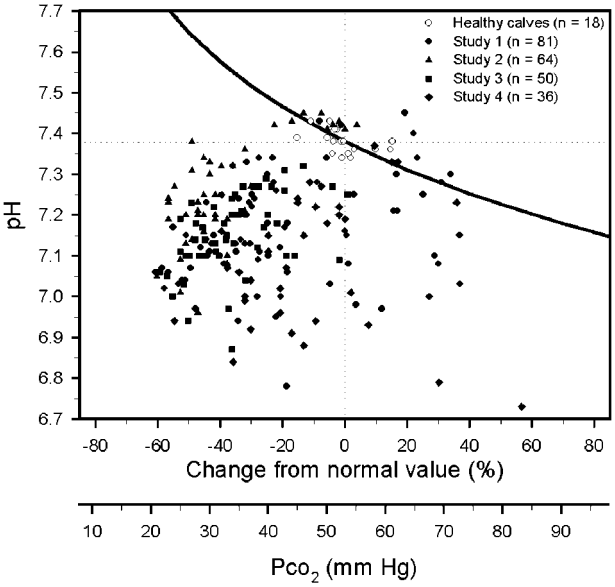

Fig. 2. Spider plot revealing the dependence of plasma pH on changes in the P_{CO_2} (solid line). The spider plot was obtained by systematically varying P_{CO_2} while holding the other 2 input variables (SID and A_{tot}, total concentration of nonvolatile weak acids) at their reference values for the plasma of healthy calves (SID, 42 mEq/L; A_{tot}, 18.5 mmol/L). The dotted lines indicate that pH = 7.38 when P_{CO_2} is at its reference value for the plasma of healthy calves (53 mm Hg). Individual data points for pH and P_{CO_2} obtained from 18 healthy calves (open circles) and 4 studies involving 231 sick calves with or without diarrhea (filled symbols) are not clustered around the pH-P_{CO_2} relationship, thereby indicating that acidemia (pH < 7.38) was not associated with respiratory acidosis.

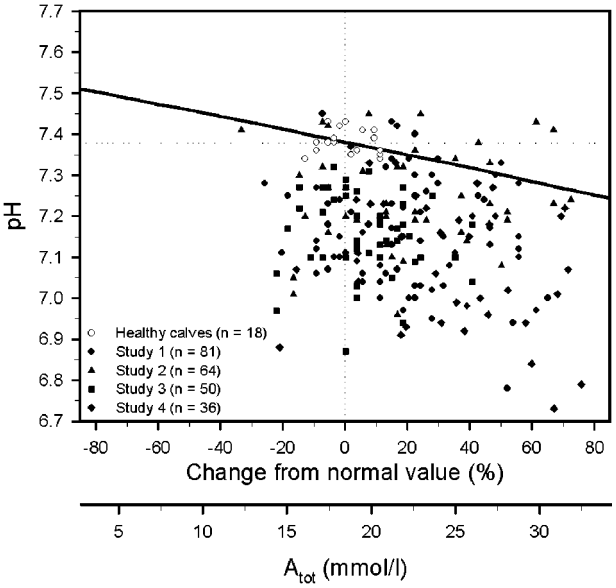

Fig. 3. Spider plot revealing the dependence of plasma pH on changes in the A_{tot} (total concentration of nonvolatile weak acids; solid line). The spider plot was obtained by systematically varying A_{tot} while holding the other 2 input variables (strong ion difference, SID; P_{CO_2}) at their reference values for the plasma of healthy calves (SID, 42 mEq/L; P_{CO_2}, 53 mm Hg). The dotted lines indicate that pH = 7.38 when A_{tot} is at its reference value for the plasma of healthy calves (18.5 mmol/L). Individual data points for pH and A_{tot} obtained from 4 studies involving 231 sick calves with and without diarrhea (filled symbols) tend to have a lower pH than the pH-A_{tot} relationship, thereby indicating that acidemia (pH < 7.38) was partially associated with nonvolatile buffer ion acidosis.

pendent component of net anion charge of protein and phosphate; $[A^-] = A_{tot}/(1 + 10^{pKa-pH}) = 18.6/(1 + 10^{7.08-7.38}) = 12.4$ mEq/L when A_{tot} is expressed in units of mmol/L, assuming HA is uncharged and A^- has a valence of -1. Because the net buffer ion charge of phosphate at pH = 7.38 approximates 1.9 mEq/L (0.8 × the calf plasma phosphate concentration of 2.4 ± 0.2 mmol/L, n = 18, unpublished data), the net buffer ion charge of protein approximates 12.4 − 1.9 = 10.5 mEq/L. As calculated previously, the pH-independent (net strong ion) charge of calf plasma protein approximates 0 mEq/L. The net plasma protein charge at pH = 7.38 is therefore approximately 10.5 mEq/L, equivalent to 0.19 mEq/g of total protein or 0.34 mEq/g of albumin.

Discussion

Use of the strong ion approach to evaluate acid-base status requires species-specific values for A_{tot} and K_a. In this study, we experimentally determined mean values for A_{tot} (0.343 mmol/g of total protein or 0.622 mmol/g of albumin) and K_a (0.84×10^{-7}) in plasma of calves. These A_{tot} and K_a values were similar to those for cattle and humans but different from those reported for horses. The finding that the A_{tot} and K_a values for bovine and human plasma are similar is consistent with observations that the pH-P_{CO_2} coordinates for the base excess curve of bovine plasma parallel those for human plasma.[37,38]

An important finding of this study is that acidemia in sick calves with or without diarrhea is due predominantly to a strong ion acidosis in response to hyponatremia accompanied by normochloremia or hyperchloremia and an increased unmeasured strong anion concentration that is presumed to be predominantly due to hyper-D-lactatemia.[9] Acidemia is due to a lesser extent to a nonvolatile buffer ion acidosis as a result of an increased plasma protein concentration. These findings confirm current recommendations that the focus of fluid therapy in acidemic diarrheic calves should be to administer an isosmotic to mildly hyperosmotic fluid containing sodium and a high effective SID,[39,40] such as 1.3% or 1.4% sodium bicarbonate solutions. The effectiveness of isosmotic sodium bicarbonate solutions in treating diarrheic calves has become widely accepted on the basis of the results of randomized, controlled clinical trials.[40] An oral electrolyte solution with a high effective SID would also appear to be more appropriate to treat acidemic diarrheic calves. This recommendation is consistent with the results of 5 studies[41–45] indicating that administration of an oral electrolyte solution with a high effective SID (79–93 mEq/L) is more effective in correcting hyponatremia and metabolic acidosis than a solution of much lower effective SID (0–31 mEq/L).

The Henderson-Hasselbalch equation has traditionally been used to guide treatment of animals with acid-base abnormalities. There are 3 reasons why the clinical applica-

tion of the Henderson-Hasselbalch equation has been successful in guiding the treatment of acid-base disturbances in sick calves with or without diarrhea. First, the treatment of animals with acidemia and low plasma [HCO_3] has focused on the intravenous administration of an isosmotic sodium bicarbonate solution, with correction of the acid-base abnormality being attributed to an increase in plasma [HCO_3] after treatment, instead of an increase in plasma SID.[46] Second, calculation of the standardized base excess or actual bicarbonate concentration has provided a quick and reliable method for calculating bicarbonate requirements, assuming that bicarbonate distributes in the extracellular fluid space.[21] Third, Equation (2) indicates that the change in plasma [HCO_3] from normal is equivalent to the change in plasma SID from normal, as long as there has been no change in plasma [A^-], and therefore the plasma albumin, globulin, and phosphate concentrations and pH. Although plasma protein concentration does vary markedly in sick calves with or without diarrhea (Fig. 3), the mean increase in plasma protein concentration (and therefore A_{tot}) was approximately 20%. Because mean pH decreased to approximately 7.15 in the calves in this study (Fig. 1), the calculated value for [A^-] in sick calves (12.0 mEq/L) was similar to that in healthy calves (12.3 mEq/L). The apparent success of plasma [HCO_3] in quantifying the magnitude of the metabolic acid-base abnormality in sick calves is therefore due to the relatively constant value for [A^-].

The traditional (Henderson-Hasselbalch) approach to acid-base balance has usually involved calculation of the anion gap (AG) in order to quantify the unmeasured anion concentration. The reference range for the AG of healthy calves (n = 18) is 8.9–15.0 mEq/L (unpublished data) using the analytical equipment used in this study. We calculated the net anion charge of protein and phosphate in plasma to be 12.4 mEq/L at normal pH. This result indicates that the AG in healthy calves approximates the net anionic charge of plasma protein and phosphate. Changes in the AG of sick calves may therefore result from changes in the plasma protein concentration, phosphate concentration, or unmeasured strong cation and anion concentrations. In other words, the AG is a nonspecific method for quantifying the unmeasured strong anion concentration (such as D-lactate or L-lactate) in calf plasma.

A clinically useful method for adjusting the AG value in calf plasma for changes in the albumin or total protein concentration is calculation of an albumin or total protein-adjusted AG,[47] whereby

$$\text{albumin-adjusted AG} = \text{AG} + 0.34 \times (31.1 - [\text{albumin}]) \quad (7)$$

and

$$\text{total protein-adjusted AG} = \text{AG} + 0.19 \times (54.1 - [\text{total protein}]), \quad (8)$$

where the albumin and total protein concentrations are measured in grams/liter and are subtracted from reference mean values for serum albumin concentration (31.1 g/L) and serum total protein concentration (54.1 g/L, unpublished observations, n = 18). Equations (7) and (8) are formulated to provide the normal reference range when albumin or total protein concentrations are normal and, therefore, are familiar to the clinician.[47] The equations can be programmed into automated clinical biochemistry analyzers that measure serum sodium, potassium, chloride, albumin, total protein, and total CO_2 concentrations, thereby providing a more accurate estimate of unmeasured strong ion charge in calf plasma than that currently provided by the AG. An albumin-adjusted or total protein-adjusted AG above the normal reference range therefore provides a more specific indicator of the presence of unmeasured strong anions than does the traditional AG.

A useful clinical application of strong ion difference theory is calculation of the strong ion gap (SIG)[24,28,48] instead of the anion gap. The SIG provides an estimate for the difference among the net strong ion charge of plasma nonvolatile buffers (albumin, globulin, and phosphate), unmeasured strong anion concentration (L-lactate, D-lactate, sulfate, nonesterified fatty acids, ketoacids, pH-independent phosphate charge, and other strong anions), and unmeasured strong cation concentrations (Ca^{2+}, Mg^{2+}). Total protein or albumin can be used to index net nonvolatile buffer ion charge; however, because albumin is the most important buffer in the plasma of humans (exerting 73% of total buffering, compared with 22% for globulin and 5% for phosphate),[49] albumin may provide the best index, assuming albumin is the most important buffer in calf plasma. On the basis of the estimated values for A_{tot} and K_a, the following equations for calculating SIG (in mEq/L) in the plasma of calves are proposed:

$$\text{SIG} = [\text{albumin}] \cdot (0.622/\{1 + 10^{[7.08-\text{pH}]}\}) - \text{anion gap}, \quad (9)$$

$$\text{SIG} = [\text{total protein}] \cdot (0.343/\{1 + 10^{[7.08-\text{pH}]}\}) - \text{anion gap}, \quad (10)$$

where [total protein] and [albumin] are in grams/liter and the anion gap (in mEq/L) = ([Na^+] + [K^+]) − ([Cl^-] + [HCO_3^-]). Applying the reference values for jugular venous plasma of healthy Holstein bull calves (n = 18; pH = 7.38; [albumin] = 31.1 g/L; [total protein] = 54.1 g/L; unpublished data) and Equations (9) and (10), the SIG is approximately −1 mEq/L (using [albumin]) and 0 mEq/L (using [total protein]). Because the reference range for AG spans approximately 6 mEq/L, the reference range for SIG should approximate 6 mEq/L. On this basis, a SIG >3 mEq/L indicates an increase in unidentified strong cations (a rare event), whereas a SIG <−3 mEq/L indicates an increase in unidentified strong anions (a common event). Equations (9) and (10) can also be applied to blood gas and serum biochemistry values of healthy calves as an adjunct quality control for assessing measurement accuracy.[o] In this case, the 95% confidence interval for the mean SIG value should include 0.

In conclusion, the results indicate that metabolic acidosis in sick calves with and without diarrhea is due to hyponatremia accompanied by normochloremia or hyperchloremia, the presence of unidentified strong anions (presumed to be primarily D-lactate), and an increase in plasma protein concentration. The most appropriate fluid for treating sick calves with metabolic acidosis should therefore contain a high sodium concentration and a high effective SID.

Footnotes

[a] Staempfli HR, Petrie JP, Lumsden JH et al. The weak acid concentration of plasma in Holstein calves. VIth Congress of the International Society of Animal Clinical Biochemistry 1994;76

[b] Constable PD, Stämpfli HR. Using the simplified strong ion approach to determine the mechanism for an acid-base disturbance in calves. Le Médecin Vétérinaire du Québec 2004;34:105 (abstract #026)

[c] Ward DE. Pathophysiology of enteric colibacillosis in the intact neonatal calf. Ithaca, NY: Cornell University, 1976. Dissertation

[d] Constable PD, Stämpfli HR. Clinical assessment of acid-base status in dogs: calculation of plasma Atot and Ka values for use in the strong ion and simplified strong ion models. J Vet Int Med 2004;18: 391 (abstract #24)

[e] Model IL235, Instrumentation Laboratory, Lexington, MA

[f] Statprofile 9+, NOVA Biomedical, Canada LTD, Mississauga, Ontario, Canada

[g] Dacos multi-analyzer, Coulter Electronics, Hialeah, FL

[h] PROC NLIN, SAS 8e, SAS Institute, Cary, NC

[i] Berchtold J, Constable P, Smith G, et al. Acid-base, cerebrospinal fluid, and cardiovascular effects of rapid intravenous hypertonic sodium bicarbonate administration in calves with experimentally induced respiratory and metabolic acidosis. J Vet Int Med 2001;15:284

[j] Hitachi 704 automatic analyzer, Hitachi, Tokyo, Japan

[k] i-STAT, i-STAT Corp, Princeton, NJ

[l] Lactate, Sigma Diagnostics Inc, St. Louis, MO

[m] PROC REG, SAS 8e, SAS Institute, Cary, NC

[n] PROC TTEST, SAS 8e, SAS Institute, Cary, NC

[o] Stämpfli, AJ Stevenson, L Brooks, MP Weber. The use of physiochemical calculations for additional quality control of automated multianalyzer and blood gas measuring systems. Proceedings XI Congress International Society of Animal Clinical Biochemistry. Chile 2004;9:66

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