



Quantitative Physicochemical Analysis of Acid-Base Balance and Clinical Utility of Anion Gap and Strong Ion Gap in 806 Neonatal Calves with Diarrhea

F.M. Trefz, P.D. Constable, and I. Lorenz

Background: Acid-base abnormalities in neonatal diarrheic calves can be assessed by using the Henderson-Hasselbalch equation or the simplified strong ion approach which use the anion gap (AG) or the strong ion gap (SIG) to quantify the concentration of unmeasured strong anions such as D-lactate.

Hypothesis/Objectives: To determine and compare the clinical utility of AG and SIG in quantifying the unmeasured strong anion charge in neonatal diarrheic calves, and to examine the associations between biochemical findings and acid-base variables by using the simplified strong ion approach. We hypothesized that the SIG provides a more accurate prediction of unmeasured strong anions than the AG.

Animals: Eight hundred and six neonatal diarrheic calves admitted to a veterinary teaching hospital.

Methods: Retrospective study utilizing clinicopathologic findings extracted from medical records.

Results: Hyperphosphatemia was an important predictor of venous blood pH. Serum inorganic phosphorus and plasma D-lactate concentrations accounted for 58% of the variation in venous blood pH and 77% of the variation in AG and SIG. Plasma D- and total lactate concentrations were slightly better correlated with SIG ($r_s = -0.69; -0.78$) than to AG ($r_s = 0.63; 0.74$).

Conclusions and Clinical Importance: Strong ion gap is slightly better at quantifying the unmeasured strong anion concentration in neonatal diarrheic calves than AG. Phosphorus concentrations should be included as part of the calculation of A_{tot} when applying the simplified strong ion approach to acid-base balance to critically ill animals with hyperphosphatemia.

Key words: D-Lactate; Henderson-Hasselbalch model; Inorganic phosphorus; Strong ion difference.

The Henderson-Hasselbalch equation has been successfully used for the diagnosis and treatment of acidemia because of metabolic (strong ion) acidosis for many decades. Metabolic acidosis and acidemia is frequently evident in neonatal calves with diarrhea and has been traditionally attributed to intestinal losses of bicarbonate and an increase in plasma L-lactate concentration as a result of dehydration and concomitant tissue hypoperfusion.^{1,2} Metabolic acidosis in diarrheic calves is usually accompanied by an increased anion gap (AG) which represents the presence of unmeasured anions in plasma. The increase in AG cannot be satisfactorily explained by increased plasma L-lactate concentrations,^{3,4} but the recent discovery that increased plasma concentrations of D-lactate play an important role in the pathogenesis of metabolic (strong ion) acido-

Abbreviations:

AG	anion gap
SIG	strong ion gap
USI	unidentified strong ions

sis in calves with diarrhea has markedly increased our understanding of the mechanism of acidemia in diarrheic calves.^{5,6} Application of the simplified strong ion approach⁷ to dehydrated diarrheic calves identified that acidemia and strong ion acidosis was primarily because of a decreased strong ion difference as a result of hyponatremia and an increase in unmeasured strong anions such as D-lactate. A nonvolatile buffer ion acidosis, because of an increase in serum protein concentration, contributed to the acidemia in dehydrated diarrheic calves.⁸

The simplified strong ion approach to acid-base balance is based on Stewart's strong ion model,^{9,10} and is conceptually different from the bicarbonate-centered Henderson-Hasselbalch approach. The simplified strong ion approach explains how three independent variables (pCO₂, the strong ion difference, and the concentration of nonvolatile weak acids such as albumin, globulin, and phosphorus) directly determine blood pH and plasma bicarbonate concentration.⁷ A useful clinical application of the simplified strong ion acid-base model involves calculation of the strong ion gap (SIG), which is defined as the difference between the concentration of unmeasured strong cations and strong anions in plasma. In contrast to the traditional AG, this approach considers the anion charge of nonvolatile weak acids as variable and has been shown to provide a more accurate prediction of the plasma concentration of strong anions in adult cattle and horses than the AG.^{11,12} This is of

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relevance because the results of retrospective and prospective studies have demonstrated that the unmeasured anion concentration, quantified by calculating the AG or SIG, had a major impact on the acid-base status in neonatal calves with diarrhea and was closely correlated with clinical alterations such as changes of posture, behavior, and suckling activity.^{6,13,14} However, to our knowledge, the determinants of AG and SIG have not been fully characterized in a large number of diarrheic calves. The aim of this study was therefore to compare the clinical utility of the AG,^{12,15} total protein-adjusted AG,⁸ SIG⁸, and the related unmeasured anion (*cXA*) approach¹⁶ in quantifying the unmeasured strong anion and D-lactate concentration in diarrheic calves. Another purpose was to examine the association between clinicopathologic findings and acid-base variables by using the simplified strong ion approach to further identify potential mechanisms for profound acid-base derangements in affected animals.

Materials and Methods

Case Selection

The medical records of 832 calves with diarrhea up to an age of 21 days admitted to the Clinic for Ruminants, LMU Munich, between April 2005 and December 2007 were reviewed. Data were retrieved from the medical record of calves admitted for treatment of diarrhea or with a clinical diagnosis of diarrhea on initial examination, provided that jugular venous blood partial pressure of carbon dioxide (*pCO*₂) and pH, and the plasma concentrations of sodium, potassium, calcium, and chloride were measured by using ion-selective potentiometry on admission and before treatment was administered. Diarrhea was defined as a fecal consistency that permitted feces to run through slightly opened fingers. Additional information retrieved from the medical records included signalment (age, sex, breed) and the results of hematologic and clinical biochemistry analysis, including plasma or serum concentrations of D-lactate, L-lactate, magnesium, phosphorus, total protein, albumin, urea, and creatinine. This dataset was used recently in a retrospective study that focused on the clinicopathologic factors associated with hyperkalemia in neonatal diarrheic calves with diarrhea.¹⁷

Laboratory Analyses

Blood pH, *pCO*₂, and plasma sodium, chloride, potassium, and ionized calcium concentrations were determined by using a blood pH, gas and electrolyte analyzer^a with ion-selective electrodes. This was performed by using lithium-heparinized blood samples that were anaerobically collected using a 2-mL polypropylene syringe. Blood pH and *pCO*₂ were corrected for rectal temperature using standard algorithms.¹⁸ Actual bicarbonate concentration (*cHCO*₃⁻) was calculated by using the Henderson-Hasselbalch equation with measured blood pH and *pCO*₂ at 37°C:

$$c\text{HCO}_3^- = S \times p\text{CO}_2 \times 10^{(\text{pH} - \text{p}K_1')} \quad (1)$$

Values used for the negative logarithm of the dissociation constant of carbonic acid (*pK*₁') and solubility of carbon dioxide (*S*) for plasma at 37°C were 6.105 and 0.0307 mmol/L per mmHg, respectively. After measuring the hemoglobin concentration (*cHb*) in mg/dL photometrically, base excess (in vitro base excess) was calculated in mmol/L with measured values for venous blood pH at 37°C and the calculated actual bicarbonate (equation 1) as:

$$\text{Base excess} = (1 - 0.014 \times c\text{Hb}) \times [(c\text{HCO}_3^- - 24.8) + (7.7 + 1.43 \times c\text{Hb}) \times (\text{pH} - 7.40)] \quad (2)$$

An automatic analyzer^b was used for serum biochemical analysis. Serum samples (plain tubes) were assayed for concentrations of urea (urease), creatinine (picric acid), total protein (biuret), albumin (bromocresol green), inorganic phosphorus (molybdenum), and total magnesium (xylydyl blue). The net charge on magnesium was calculated from the total magnesium concentration assuming that 69% of total magnesium was in the ionized form.¹⁹ Concentrations of D-lactate and L-lactate were determined from blood samples containing lithium heparin and potassium fluoride as a glycolytic agent. Concentrations of D- and L-lactate were determined photometrically by means of enzymatic methods using D- and L-lactate dehydrogenase.^{20,21}

Methods for Estimating the Unmeasured Strong Anion Concentration

Four different methods for estimating the unmeasured strong anion concentration were compared. These four methods were the traditional AG,^{12,15} the total protein-adjusted AG,⁸ the SIG⁸, and the related Figge et al unmeasured anion (*cXA*) approach.¹⁶ The AG, which is defined as the difference between unmeasured cations and unmeasured anions, was calculated in mEq/L as follows^{12,15}:

$$\text{AG} = (c\text{Na}^+ + c\text{K}^+) - (c\text{Cl}^- + c\text{HCO}_3^-) \quad (3)$$

The calculated values for AG can be adjusted for changes in the albumin or total protein concentration.^{8,22} We elected to adjust the AG for the total protein concentration for three reasons. First, globulin does not appear to carry a net charge in human plasma,^{16,22,23} whereas studies in domestic animals have shown that globulin carries a net negative charge.^{7,8,12,15} Second, the plasma concentration of albumin and total protein are measured to one decimal place yet the value for total protein is approximately twice that of albumin. As a result, the estimate of net negative charge is more precise when total protein is used because the effect of rounding error is decreased. Third, total protein was used to permit comparison between AG and SIG, as the latter factor is calculated on the basis of total protein concentration.

Using the calculated value for net protein charge of 0.19 mEq/g total protein and reported reference values for 18 healthy Holstein-Friesian calves for total protein (54.1 g/L), the total protein-adjusted AG was calculated in mEq/L as follows:⁸

$$\text{Total protein-adjusted AG} = \text{AG} + 0.19 \times (54.1 - [\text{measured total protein}]) \quad (4)$$

In contrast to the traditional Henderson-Hasselbalch equation, the simplified strong ion model involves calculation of the SIG which is defined as the difference between unmeasured strong cations and unmeasured strong anions, where strong cations and anions are completely dissociated at physiologic pH. The value for SIG in mEq/L was calculated using the experimentally determined value for *A*_{tot}, the experimentally determined value for the negative logarithm of dissociation constant of plasma nonvolatile weak acids (*pK*_a = 7.08), and the following equation, whereby:⁸

$$\text{SIG} = A_{\text{tot}} / (1 + 10^{(7.08 - \text{pH})}) - \text{AG} \quad (5)$$

An alternative method for calculating the SIG in human plasma was reported by Kellum et al²³ that utilized the Figge et al [XA]

approach.¹⁶ They defined the SIG as the difference between the apparent strong ion difference (net charge difference between sodium, potassium, magnesium, and calcium ions on one hand and chloride ions on the other hand) and the effective strong ion difference (defined as the anion charge of bicarbonate, phosphorus, and protein) such that:

$$cXA = cNa^+ + cK^+ + cMg^{2+} + cCa^{2+} - cCl^- - cHCO_3^- - (\text{protein charge}) - (\text{phosphorus charge}). \quad (6)$$

As the originally reported calculations for protein charge of human plasma¹⁶ are not applicable to bovine plasma, the charge of albumin and globulin was calculated according to Darrow and Hartmann²⁴ as suggested by Constable¹¹ with albumin and globulin expressed in g/L:

$$\text{Albumin charge} = (0.141 \times [\text{albumin}]) \times (\text{pH} - 5.42), \quad (7)$$

$$\text{Globulin charge} = (0.04 \times [\text{globulin}]) \times (\text{pH} - 5.58). \quad (8)$$

The charge of phosphorus was calculated as suggested by Figge et al¹⁶ with phosphorus concentration expressed in mmol/L:

$$\text{Phosphorus charge} = [\text{phosphorus}] \times (0.309 \times \text{pH} - 0.469). \quad (9)$$

Variables of the Simplified Strong Ion Acid-Base Model

The strong ion difference in mEq/L calculated from the plasma concentrations of sodium, potassium, and chloride was obtained as follows⁸:

$$SID_3 = cNa^+ + cK^+ - cCl^-. \quad (10)$$

The measured strong ion difference obtained from seven strong ions²⁵ (SID_7 , mEq/L) was calculated using the measured value for $[Ca^{2+}]$ determined by ion-selective potentiometry and assigning a charge of +1.38 to magnesium assuming 69% dissociation¹⁹ and -1 to D-lactate and L-lactate assuming 100% dissociation such that:

$$SID_7 = cNa^+ + cK^+ + cMg^{2+} + cCa^{2+} - cCl^- - c(L - \text{lactate}) - c(D - \text{lactate}). \quad (11)$$

The concentration of nonvolatile weak acids (A_{tot}) in mmol/L in calf plasma was calculated from serum concentrations of total protein⁸:

$$A_{tot} = 0.343 \times c(\text{total protein}). \quad (12)$$

Calculated values for SIG were corrected for the measured concentrations of D- and L-lactate and Ca^{2+} and Mg^{2+} to obtain the concentration of still unidentified strong ions (USI) in mEq/L for the calculation of the effective strong ion difference (SID_{eff})²⁵:

$$USI = SIG + c(D - \text{lactate}) + c(L - \text{lactate}) - cMg^{2+} - cCa^{2+}, \quad (13)$$

$$SID_{eff} = SID_7 + USI = SID_3 + SIG. \quad (14)$$

Statistical Analysis

Values of $P < .01$ were considered to be statistically significant because of the relatively large dataset and number of comparisons. Data are presented as medians and interquartile ranges (Q_{25}/Q_{75}) because most of the data were not normally distributed, based on Shapiro-Wilk W test and visual examination of QQ-plots.

Spearman's correlation coefficient (r_s) was calculated to characterize associations between parameters. Stepwise forward multivariable regression models were constructed, including measured variables of clinical pathology significantly correlated with the dependent variables or considered biologically relevant. To minimize the effects of collinearity and ensure an appropriately low variance inflation factor for individual values,²⁶ when two variables were closely correlated with each other ($r_s > 0.70$), only the variable that had the highest r_s for blood pH was entered into the model. The relative importance of the included variables was assessed by the order of entry into the model as well as by the change in the model R^2 value (ΔR^2). Studentized residual plots of each multivariable model and partial models and normality plots were examined to confirm an approximately normal distribution of residuals, the absence of outliers, linearity of the response, and the absence of heteroscedasticity.²⁶ Independent variables were log-transformed to the base 10 when indicated by their mathematical relationship with the dependent variable (as stated in equations 1–6) or whenever indicated by examination of student residual plots, normality plots, variance inflation factor, and examination of the linearity of partial model plots. All interaction terms were investigated for each significant predictor in multivariable models; however, in all cases, the addition of interaction terms failed to materially improve the model fit and made it difficult to interpret coefficient values.²⁶ Moreover, the variance inflation factor for all predictors were <6 ; multicollinearity is typically considered a concern when the variance inflation factor exceeds 10.²⁶

Based on the nonlinear association between pH and $cHCO_3^-$ in the Henderson-Hasselbalch equation (equation 1) and the linear relationship in equation 3, nonlinear regression was used to characterize the relationship between venous blood pH and AG, such that:

$$\text{pH} = \log_{10}(a - \text{AG}) + b. \quad (15)$$

Equation 15 was derived from algebraic rearrangement of equations of 1 and 3, such that:

$$\text{pH} = \log_{10}(SID_3 - \text{AG}) + \{pK'_1 - \log_{10}(S \times pCO_2)\}. \quad (16)$$

Similarly, based on the relationships in equations 1 and 5, nonlinear regression was used to characterize the relationship between venous blood pH and SIG, such that:

$$\text{pH} = \log_{10}(\text{SIG} + c) + d. \quad (17)$$

This equation was derived from algebraic rearrangement of equations of 1 and 5, assuming that the net nonvolatile buffer ion charge (A^-) can be expressed as $A_{tot}/(1 + 10^{(7.08 - \text{pH})})$:

$$\text{pH} = \log_{10}(\text{SIG} + SID_3 - A_{tot}/(1 + 10^{(7.08 - \text{pH})})) + \{pK'_1 - \log_{10}(S \times pCO_2)\}. \quad (18)$$

A pseudo- R^2 value was calculated from the results of nonlinear regression, whereby: pseudo- $R^2 = 1 - \text{SS}(\text{Residual})/\text{SS}(\text{Total Corrected})$. The software package SPSS 18.0 for Windows^c and SAS 9.3^d was used for statistical analysis.

Results

Availability of results of clinical biochemistry analysis allowed calculation of USI, SID_7 , SID_{eff} , and cXA in a total of 820 calves. Multivariate regression analysis identified that data points from 14 of the 820 calves were statistical outliers, based on student residual values >3.5 or <-3.5 . Consequently, data from 806 calves were

used in the analysis reported here. Because of regional preferences, most of the calves (93%) were Simmental (German Fleckvieh), which is the most common dairy breed in Bavaria.

Median and interquartile ranges for selected laboratory variables of calves of the study population are shown in Table 1. The median and interquartile range for age was 9.4 (7.0–12.4) days. The median value for SIG (−11.6 mEq/L) indicated that unmeasured strong anion concentration approximated the calculated value for cXA (11.2 mEq/L). Spearman's correlation coefficients between clinicopathologic factors and variables of acid-base status can be found in Table S1; values for $r_s > 0.70$ were identified for the relationships between serum inorganic phosphorus and serum magnesium, creatinine, and urea concentrations, and between plasma sodium and chloride concentration. Phosphorus, urea, sodium, and chloride were retained in dataset used for multivariate analysis; urea was retained as an index of glomerular filtration rate and both sodium and chloride were retained because the difference between their concentrations is a major independent determinant of blood pH. The quantified unmeasured anion (AG, total protein-adjusted AG) and strong anion concentrations (SIG, cXA) were closely correlated with venous blood pH, actual bicarbonate concentration, and base excess with r_s values being consistently higher for SIG and cXA than AG and total protein-adjusted AG (Table 2).

Venous blood pH values in relation to the calculated values of AG and SIG are depicted in Figures 1 and 2. The nonlinear equation relating pH to AG was:

$$pH = \log_{10}(39.7 - AG) + 5.92. \quad (19)$$

The pseudo- R^2 value for equation 19 was 0.62. The 95% confidence interval of the estimate for a in equation 15 was 38.6 to 40.8; this included the median value for SID_3 (38.7 mEq/L, Table 1).

The nonlinear equation relating pH to SIG was:

$$pH = \log_{10}(SIG + 32.4) + 5.84. \quad (20)$$

The pseudo- R^2 value for equation 20 was 0.72, indicating that the pH-SIG relationship had greater explanatory power than the pH-AG relationship.

Scatterplots of venous blood pH and the independent variables of the simplified strong ion acid-base model (A_{tot} , phosphorus concentration, pCO_2 , and SID_{eff}) are depicted in Figure 3. A strong curvilinear association ($r_s = 0.92$, $P < .001$) was observed between venous blood pH and SID_{eff} . The Spearman's correlation coefficients between blood pH and serum phosphorus concentration, pCO_2 , and A_{tot} were −0.53, 0.50, and −0.29, respectively.

To explore the importance of the SID_7 , A_{tot} , and pCO_2 in the development of acid-base derangements in neonatal diarrheic calves, those variables were included in three multivariable linear regression models that aimed to predict venous blood pH (as $\log_{10}SID_7$, $\log_{10}A_{tot}$, and $\log_{10}pCO_2$, based on the mathematical

relationships in equations 16, 17, and 18), $cHCO_3^-$, and base excess as dependent variables (Table 3). The $\log_{10}SID_7$ and SID_7 had the highest explanatory power in the model for venous blood pH and base excess, whereas $cHCO_3^-$ was best predicted by pCO_2 . The final models explained 47, 71, and 63% of the variation in venous blood pH, actual $cHCO_3^-$, and base excess, respectively. To further investigate associations with venous blood pH and strong ion difference, the \log_{10} values for specific strong ions such as D-lactate, L-lactate, inorganic phosphorus (which has a strong ion and buffer ion component to its charge), sodium, chloride, calcium, and potassium, as well as total protein and pCO_2 , were entered into a multivariable stepwise linear regression model (Table 4). The final model explained 75% of the variation in venous blood pH. Plasma D-lactate and serum phosphorus concentrations had the highest explanatory power and accounted for 38% and 18% of the variation in venous blood pH, respectively.

Spearman's coefficients of correlation between selected laboratory variables and the quantified unmeasured anion and strong anion concentration by means of AG, total protein-adjusted AG, SIG, and cXA are presented in Table 5. D-lactate and total lactate concentrations were most closely correlated with calculated cXA . The inorganic phosphorus concentration was most closely correlated with AG. Only cXA was significantly, but negatively, associated with L-lactate concentrations. To identify important contributors to AG, total protein-adjusted AG, SIG, and cXA , multivariable stepwise linear regression models were constructed including plasma or serum concentrations of D-lactate, L-lactate, inorganic phosphorus, urea, albumin, and globulin as independent variables (Table 6). Inorganic phosphorus concentration had the highest explanatory power in the model for AG, whereas plasma D-lactate concentration had the highest explanatory power in the models for total protein-adjusted AG, SIG, and cXA . For the analyses in Table 6, only urea was log-transformed because regression diagnostics indicated that this provided a better model fit. All other variables in Table 6 were not transformed for analysis because they were linearly related to the dependent variable. Phosphorus was not included in the model for cXA , as cXA includes phosphorus in its calculation.

Discussion

The results of this retrospective study indicate that the simplified strong ion approach has many advantages over the traditional Henderson-Hasselbalch model in explaining the mechanisms for acid-base derangements in calves with neonatal diarrhea. One marked disadvantage of the Henderson-Hasselbalch model is that it ignores the effects of strong ions (eg, sodium and chloride) and nonvolatile weak acids such as albumin or inorganic phosphate on plasma pH.²⁷ Scatterplots of calculated A_{tot} values against venous blood pH and linear regression analysis revealed a negative association between those parameters (Fig 3) which reinforces the concept that dehydration and concomitant hemoconcen-

Table 1. Median and interquartile range (Q_{25}/Q_{75}) of selected laboratory variables in 806 neonatal calves with diarrhea.

Variable	Median	Q_{25}/Q_{75}	Reference Values
<i>Henderson-Hasselbalch model</i>			
Venous blood pH	7.16	7.01/7.31	[7.35 to 7.50] ³⁸
pCO ₂ (mmHg)	47.1	37.3/55.4	[34 to 45] ³⁸
HCO ₃ ⁻ (mmol/L)	15.8	9.8/26.4	[20 to 30] ³⁸
Base Excess (mmol/L)	-12.0	-20.2/-0.6	[-3.5 to 3.5] ³⁹
AG (mEq/L)	22.3	13.3/27.8	[8.9 to 15.0] ⁸
Total protein-adjusted AG	21.2	13.3/26.9	n.a.
<i>Strong ion difference model</i>			
SID ₃ (mEq/L)	38.7	34.5/42.9	[38.3 to 47.7] ⁸
SID ₇ (mEq/L)	34.8	27.9/40.3	n.a.
SID _{eff} (mEq/L)	27.0	19.0/37.9	[37.3 to 51.5] ⁸
USI (mEq/L)	-5.7	-10.5/-0.2	n.a.
A _{tot} (mmol/L)	19.7	17.2/22.2	[15.9 to 21.2] ⁸
A ⁻ (mEq/L)	10.6	9.1/12.2	n.a.
SIG (mEq/L)	-11.6	-18.1/-2.1	[-3.0 to 3.0] ⁸
cXA (mEq/L)	11.2	2.9/16.9	n.a.
<i>Clinical biochemistry analysis</i>			
D-Lactate (mmol/L)	4.2	0.8/10.4	[≤4.0] ²¹
L-Lactate (mmol/L)	1.7	1.0/3.0	[0.6 to 2.2] ³⁸
Total protein (g/L)	57.3	50.2/64.7	[40.9 to 69.1] ⁴⁰
Inorganic phosphorus (mmol/L)	3.1	2.5/4.0	[2.3 to 3.5] ⁴⁰
Urea (mmol/L)	13.4	7.6/22.4	[≤10.8] ⁴⁰
Creatinine (μmol/L)	148	105/301	[≤159] ⁴⁰
<i>Electrolytes</i>			
Sodium (mmol/L)	135	130/142	[132 to 152] ³⁸
Potassium (mmol/L)	4.9	4.3/6.0	[3.9 to 5.8] ³⁸
Chloride (mmol/L)	101	96/108	[95 to 110] ³⁸
Magnesium (mmol/L)	0.98	0.84/1.25	[0.74 to 1.10] ³⁸
Ionized Calcium (mmol/L)	1.22	1.15/1.32	[1.2 to 1.6] ³⁸

n.a., not available; pCO₂, partial pressure of carbon dioxide; AG, anion gap; SID₃, strong ion difference calculated from three strong cations and anions; SID₇, strong ion difference calculated from seven strong cations and anions; SID_{eff}, effective strong ion difference; USI, concentration of unidentified strong ions; A_{tot}, concentration of nonvolatile weak acids; A⁻, total net anion charge of nonvolatile weak acids; SIG, strong ion gap; cXA, unmeasured strong anion concentration according to Figge et al¹⁶

Table 2. Spearman's correlation coefficients between the estimated strong anion concentration by means of the anion gap, total protein-adjusted anion gap, strong ion gap, and the unmeasured strong anion concentration according to Figge et al¹⁶ (cXA), and venous blood pH, cHCO₃⁻, and base excess in 806 neonatal calves with diarrhea.

Variable	Venous Blood pH	cHCO ₃ ⁻	Base Excess
Anion gap	-0.76**	-0.72**	-0.75**
Total protein-adjusted anion gap	-0.74**	-0.72**	-0.75**
Strong ion gap	0.83**	0.79**	0.82**
cXA	-0.80**	-0.80**	-0.82**

**P < .001.

tration are associated with a decrease in plasma pH.⁸ This is compatible with clinical studies that reported a significant correlation between measures of clinical dehydration and the acid-base status of diarrhetic calves.^{13,28}

The calculated values for SID₇ (which includes the concentrations of D- and L-lactate) were only moderately correlated with measured pH ($r_s = 0.57$). However, strong ion difference has two components: measured and unmeasured strong ion difference. The latter is usually obtained by calculating the SIG, which was corrected for the strong ion charge of D-lactate, L-lactate, magnesium, and calcium in the present study to obtain the concentration of unidentified strong ions. The obtained effective strong ion difference (SID₇ + USI) was well correlated with venous blood pH with a non-linear relationship as previously reported,⁸ indicating that acidemia in neonatal diarrhetic calves is predominantly the result of a strong ion acidosis.

The relative contribution of log₁₀ values for SID₇, pCO₂, and A_{tot} in determining the acid-base balance in diarrhetic calves was assessed by means of a multivariable linear regression analysis which indicated that those independent variables predict most of the change in venous blood pH and calculated bicarbonate concentration, similar to previous reports.^{8,14} Of interest is our finding that the plasma inorganic phosphorus concentration explained 18% of the change in venous blood pH, separate to that explained by SID₇, pCO₂, and A_{tot}.

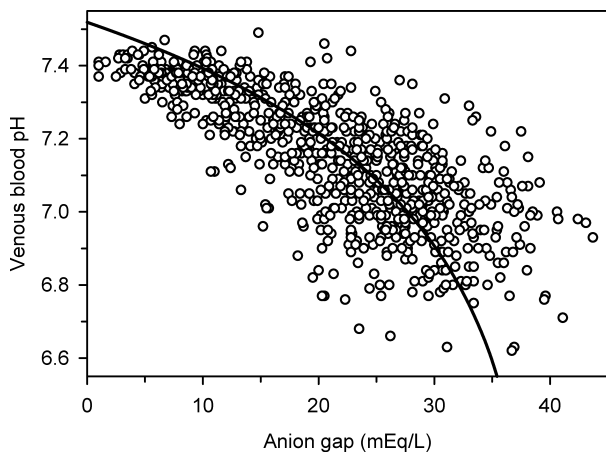


Fig 1. Relationship between venous blood pH and anion gap in 806 neonatal calves with diarrhea. The thick line represents the result of nonlinear regression analysis.

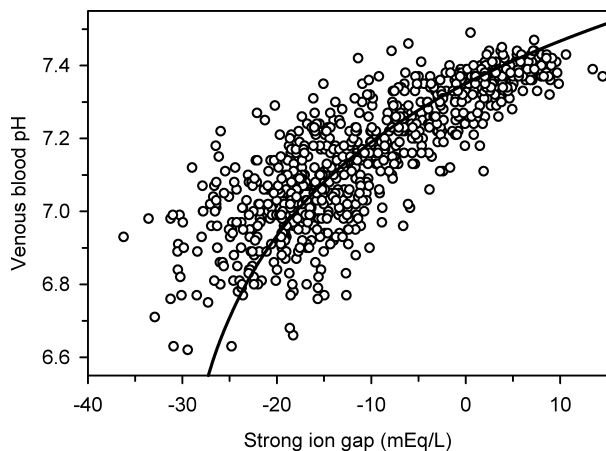


Fig 2. Relationship between venous blood pH and strong ion gap in 806 neonatal calves with diarrhea. The thick line represents the result of nonlinear regression analysis.

Similarly to dehydrated adult cattle with right sided displacement of the abomasum or abomasal volvulus,²⁹ close correlations between plasma phosphorus concentration and parameters of hydration status and renal function such as serum urea concentration ($r_s = 0.73$), creatinine concentration ($r_s = 0.74$), and magnesium concentration ($r_s = -0.77$) were found in the study reported here. This suggests that the accumulation of unmeasured strong anions in azotemic animals, such as sulfate, urate, oxalate, hippurate, and other strong anions in humans,³⁰ also play a role in the development of strong ion (metabolic) acidosis in dehydrated calves with diarrhea. A recent study³¹ also reported an unknown compound of metabolic acidosis in calves with naturally acquired diarrhea or experimentally induced acidemia, but failed to identify the compound by means of a high-performance liquid chromatography technique.

Interestingly, the results of multivariable stepwise regression analysis presented in Table 4 identify that the

coefficient for the strong anions chloride and D-lactate were -0.016 , and the coefficient for the strong cation sodium was $+0.013$. The absolute estimates for the coefficient are similar to those calculated for $dpH/d\text{Strong ion difference}$ ($+0.013$ pH units/(mEq/L) in calf plasma.⁸ Interestingly, the estimated coefficient for phosphorus was -0.067 ; based on a mean phosphorus charge of 1.8 at normal plasma pH the coefficient would be predicted to be -0.023 (-1.8×0.013). This finding provides strong support for the presence of unidentified anions that are correlated with serum inorganic phosphate concentration; it is therefore likely that uremic anions such as sulfate are present in quantitatively important amounts in dehydrated calves with diarrhea. This supposition awaits experimental confirmation.

The multivariable stepwise regression analysis identified D-lactate as the most important predictor of acidemia in diarrheic calves, which emphasizes the importance of the finding that hyper-D-lactatemia, as a result of maldigestion and malabsorption in the gastrointestinal tract and subsequent bacterial fermentation in the large intestine, plays a predominant role in the pathogenesis of metabolic (strong ion) acidosis in neonatal diarrheic calves.^{5,6,32-34} Plasma D-lactate concentration, together with sodium and chloride concentration, accounted for 51% of the variation in venous blood pH. This indicates that hyponatremia, which is probably the result of intestinal sodium losses and decreased milk intake, together with normo- or hyperchloremia as well as varying degrees of hyper-D-lactatemia, decrease the strong ion difference and therefore predominantly influence the acid-base status of diarrheic calves.⁸

The strong ion difference theory has some commonalities with the traditional bicarbonate-centered approach, and this is the reason why the Henderson-Hasselbalch model works well clinically.^{8,27} The calculated base excess values in the Henderson-Hasselbalch model are usually used to determine sodium bicarbonate requirements by multiplying the respective base deficit with body weight and a factor of 0.6–0.8 which was traditionally regarded to reflect the distribution space of bicarbonate.^{4,34} Remarkably, studies report a large individual variability when retrospectively calculating the size of this dosage factor and found a positive correlation between the factor and measured D-lactate concentrations.³⁴ This could be explained by the fact that a change in SID_7 (eg, for reasons of hyper-D-lactatemia) is equivalent to the change in base excess as long as there are normal concentrations of nonvolatile weak acids such as phosphate or proteins.²⁷ However, in cases of a nonvolatile weak acid acidosis the resulting base excess value is additionally influenced by an increase in plasma concentrations of total protein or phosphorus which can be effectively decreased by plasma volume expansion, whereas the correction of a strong ion acidosis depends on the effective strong ion difference of the provided oral or intravenous fluid solution.^{25,35}

Multivariable stepwise regression analysis indicated that the inorganic phosphorus concentration was the

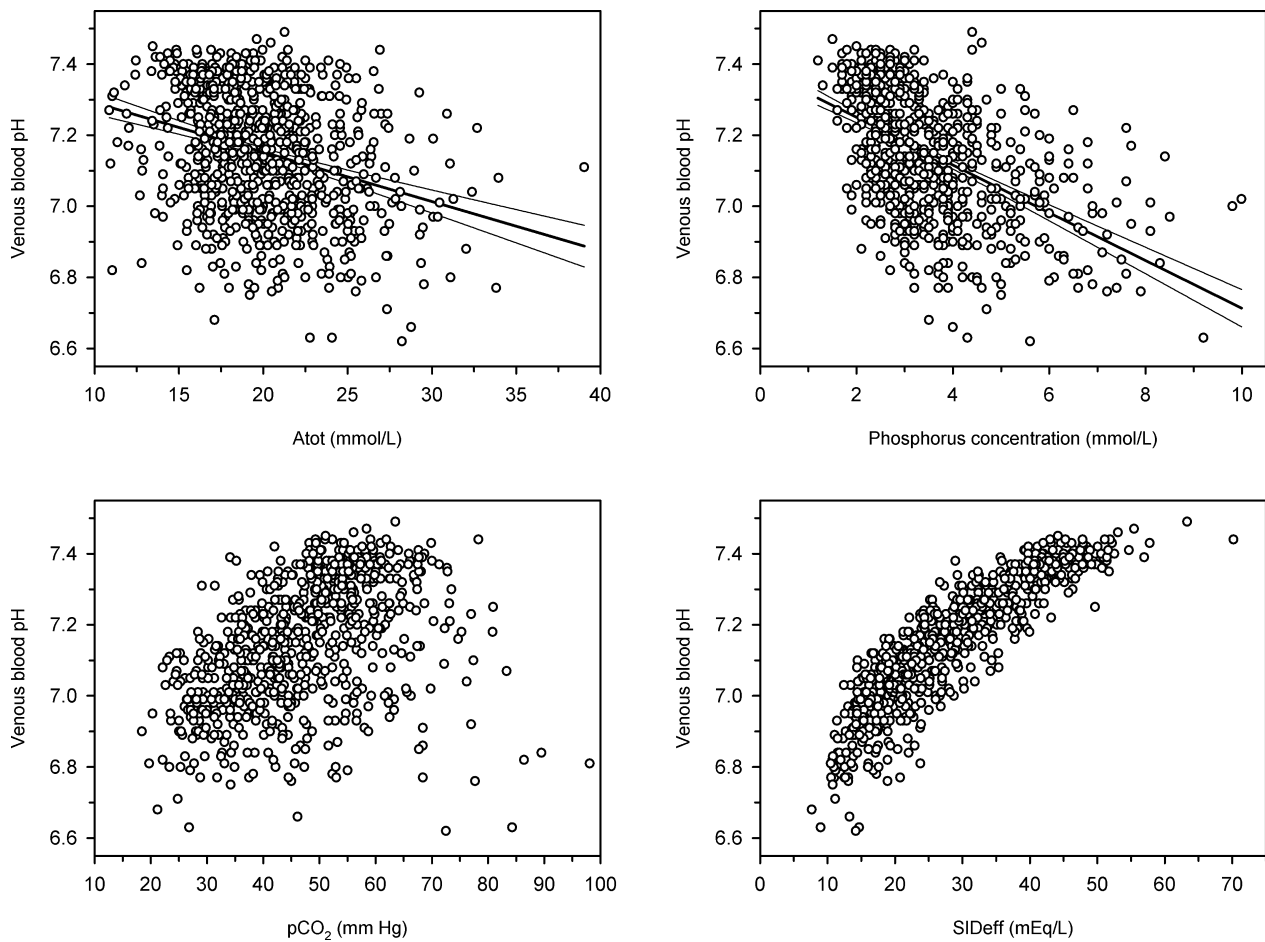


Fig 3. Scatterplots of the relationship between venous blood pH and concentrations of nonvolatile weak acids (A_{tot}), phosphorus, partial pressure of CO_2 , and effective strong ion difference (SID_{eff}) in 806 neonatal calves with diarrhea. The solid lines represent the results of linear (A_{tot} , phosphorus) regression analysis. Dashed lines indicate the 95% CI of the slope estimate.

Table 3. Results of a stepwise linear regression analysis for predicting venous blood pH, $cHCO_3^-$, and base excess (dependent variables) by means of the measured strong ion difference calculated from seven strong cations and anions (SID_7), the partial pressure of CO_2 (pCO_2), and the concentration of nonvolatile weak acids (A_{tot}) in 806 neonatal calves with diarrhea.

Order of Entry	Variable	ΔR^2	Model R^2	Variance Inflation Factor
<i>Venous blood pH as dependent variable</i>				
1	$\log_{10}SID_7$	0.314	0.314	1.03
2	$\log_{10}A_{tot}$	0.157	0.471	1.03
3	$\log_{10}pCO_2$	n.s.	0.471	—
<i>Actual HCO_3^- as dependent variable</i>				
1	pCO_2	0.534	0.534	2.15
2	SID_7	0.081	0.615	2.21
3	A_{tot}	0.095	0.710	1.04
<i>Base Excess as dependent variable</i>				
1	SID_7	0.458	0.458	2.21
2	A_{tot}	0.140	0.598	1.04
3	pCO_2	0.030	0.628	2.15

n.s., not significant.

most important predictor for an increase in AG as it was also reported for healthy calves.¹⁵ In addition, D-lactate and inorganic phosphorus concentration together explained 77% of the variation in AG and SIG. The close correlation between SIG and the phosphorus concentration was not the expected finding and might be related to inaccuracies in the calculation of the anion charge of nonvolatile weak acids which is derived from the total protein concentration. Although validated,⁸ this method represents an oversimplification of the real situation as it handles the concentrations of albumin, globulin, and phosphorus as a single nonvolatile weak acid with a single dissociation constant. However, in this context, we should be aware that inorganic phosphorus does not exclusively act as a nonvolatile weak acid because the monohydrogen phosphate compound represents a strong anion.^{7,36}

The first method for calculating SIG was reported in 1995²³ by using the *cXA* approach.¹⁶ The *cXA* approach differs from the specific SIG approach, that was used in the study reported here, in such that the phosphorus and albumin concentrations are modeled separately (based on computer modeling of ionizable

Table 4. Results of a stepwise multiple linear regression model for the prediction of venous blood pH in 806 neonatal calves with diarrhea using raw and log₁₀-transformed variables.

Order of Entry	Variable	ΔR^2	Model R^2	Coefficient	\pm SE	P-Value	Variance Inflation Factor
	Constant	—	—	7.34	0.22	<.001	—
1	log ₁₀ D-Lactate	0.380	0.380	-0.093	0.006	<.001	1.35
2	log ₁₀ Phosphorus	0.176	0.556	-0.648	0.023	<.001	1.27
3	log ₁₀ Chloride	0.064	0.620	-3.03	0.13	<.001	3.51
4	log ₁₀ Sodium	0.111	0.731	3.02	0.17	<.001	3.10
5	log ₁₀ Calcium	0.016	0.747	0.059	0.006	<.001	1.16
	Constant	—	—	7.70	0.05	<.001	—
1	D-Lactate	0.325	0.325	-0.016	0.001	<.001	2.37
2	Phosphorus	0.254	0.579	-0.067	0.003	<.001	1.77
3	Chloride	0.064	0.643	-0.016	0.001	<.001	5.72
4	Sodium	0.118	0.761	0.013	0.001	<.001	5.19
5	Calcium	0.016	0.777	-0.040	0.010	<.001	1.42
6	Total protein	0.010	0.787	-0.009	0.002	<.001	1.94
7	L-Lactate	0.006	0.792	-0.002	0.001	<.001	1.62

SE is the standard error of the coefficient of determination.

Table 5. Spearman's correlation coefficients for the relationship between selected clinicopathologic variables and the estimated unmeasured strong anion concentrations in 806 neonatal calves with diarrhea.

Variable	Anion Gap	TP-Adjusted AG	Strong Ion Gap	cXA Approach
Total lactate	0.74**	0.78**	-0.78**	0.82**
D-Lactate	0.63**	0.68**	-0.69**	0.75**
L-Lactate	0.04 ^{NS}	-0.03 ^{NS}	0.05 ^{NS}	-0.14**
Phosphorus	0.71**	0.63**	-0.65**	0.53**
Albumin	0.38**	0.24**	-0.28**	0.19**
Globulin	0.33**	0.13**	-0.16**	0.19**
Urea	0.58**	0.54**	-0.55**	0.47**
Creatinine	0.54**	0.46**	-0.47**	0.39**

** $P < .001$; ^{NS}not significant.

groups of human albumin). Unfortunately, the 1995 calculations cannot be applied to bovine plasma because bovine globulins carry also a net anion charge,²⁴ which is different from the situation in humans.¹⁶ The charge of serum albumin and globulin was therefore calculated using equations that were derived from the published results of a 1929 study reporting titration experiments of bovine albumin and globulin containing solutions.²⁴ Although the same approach was reported to be inferior to SIG in predicting plasma L-lactate concentrations in adult cattle with abomasal volvulus,¹¹ we found that cXA had the best correlation with D-lactate and total lactate. This finding might be explained by the fact that this method was the only one that separately considered the anion charge of albumin, globulin, and phosphorus concentrations. However, cXA was still moderately correlated with phosphorus concentration which indicates inaccuracies in the performed calculations or collinearity to other unidentified anions as it would likely be the case for uremic anions such as sulfate. The latter seems to be a plausible explanation based on the close correlation between serum concentrations of inorganic phosphorus and creatinine.

The measured L-lactate concentrations in calves of the present study population were not significantly correlated with the unmeasured anion concentration, quantified by calculating the AG, total protein-adjusted AG, and SIG. Similar observations were also made for AG in previous studies,^{6,37} which suggests that L-lactate plays a minor role in the development of strong ion acidosis if a considerable number of neonatal diarrheic calves are assessed.

In agreement to previous studies,^{6,37} our results demonstrate that the AG represents a clinically useful indicator of unmeasured strong anions such as D-lactate in diarrheic calves, as AG is easily derived from the results of blood gas, pH, and electrolyte analysis and does not require the determination of total protein concentration. Furthermore, calculation of a total protein-adjusted AG revealed only small advantages over the normal AG and was inferior to SIG.

Although our study provided valuable information in respect to the utility of the Henderson-Hasselbalch and simplified strong ion approaches in the assessment of acid-base abnormalities in neonatal diarrheic calves, our analyses have some potential limitations. The most prominent limitation is that multivariable linear regression analyses are prone to confounding and interaction effects. These effects were minimized in our study by removal of highly correlated variables based on $r_s > 0.70$ and the use of stepwise regression; the values for the variance inflation factors in the final regression models confirmed the absence of statistically significant confounding.

Conclusions

Our results indicate that the anion and strong ion gap approaches are similarly effective in quantifying the unmeasured strong anion concentration in neonatal diarrheic calves; however, strong ion gap had a greater explanatory power based on a numerically higher R^2 value. This result appears to be related to our finding that a hyperphosphatemic acidosis is frequently evident

Table 6. Multivariate linear regression equations showing the relationship between the estimated strong anion concentration by means of the anion gap, total protein-adjusted anion gap, strong anion gap and the *cXA* approach (dependent variables), and clinicopathologic variables in 806 neonatal calves with diarrhea.

Order of Entry	Variable	ΔR^2	Model R^2	Coefficient	\pm SE	<i>P</i> -value	Variance Inflation Factor
<i>Anion gap</i>							
	Constant	–	–	–9.70	1.03	<.001	–
1	Phosphorus	0.447	0.447	2.27	0.16	<.001	2.59
2	D-Lactate	0.320	0.767	1.00	0.03	<.001	1.26
3	log ₁₀ Urea	0.030	0.797	6.63	0.54	<.001	1.65
4	L-Lactate	0.022	0.819	0.69	0.07	<.001	1.69
5	Globulin	0.009	0.828	0.09	0.02	<.001	1.21
6	Albumin	0.005	0.833	0.18	0.04	<.001	1.46
<i>Total protein-adjusted anion gap</i>							
	Constant	–	–	–1.88	0.51	<.001	–
1	D-Lactate	0.407	0.407	1.00	0.03	<.001	1.24
2	Phosphorus	0.333	0.740	2.03	0.15	<.001	2.15
3	log ₁₀ Urea	0.033	0.773	6.63	0.55	<.001	1.64
4	L-Lactate	0.024	0.797	0.67	0.07	<.001	1.68
<i>Strong ion gap</i>							
	Constant	–	–	16.21	0.56	<.001	–
1	D-Lactate	0.419	0.419	–1.17	0.03	<.001	1.24
2	Phosphorus	0.354	0.772	–2.67	0.16	<.001	2.15
3	log ₁₀ Urea	0.034	0.806	–7.78	0.61	<.001	1.64
4	L-Lactate	0.015	0.821	–0.63	0.08	<.001	1.68
<i>cXA approach</i>							
	Constant	–	–	–10.10	0.55	<.001	–
1	D-Lactate	0.511	0.511	1.16	0.03	<.001	1.20
2	log ₁₀ Urea	0.193	0.704	9.83	0.49	<.001	1.11
3	L-Lactate	0.060	0.764	0.92	0.06	<.001	1.26

in dehydrated neonatal diarrheic calves which accounts for, in addition to D-lactate, the increase in the unmeasured plasma anion concentration, quantified by calculating the AG or SIG. Application of the simplified strong ion approach appears helpful in explaining the mechanisms responsible for the changes in acid-base status of diarrheic calves. However, the results of this study strongly indicate that compartmentalizing the concentrations of nonvolatile weak acids into albumin, globulin, and most importantly inorganic phosphorus components would probably increase the accuracy of this acid-base model when applied to animals with hyperphosphatemia.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Footnotes

^a Rapidlab® 865 blood gas analyzer, Bayer Vital GmbH, Fernwald, Germany

^b Automatic Analyzer Hitachi 911, Roche Diagnostics, Indianapolis, IN

^c IBM, New York

^d SAS 9.3, SAS Inc., Cary, NC

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Spearman's coefficients of correlation between selected clinicopathologic variables in calves of the study population.