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

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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 \( \text{\textalpha}-\text{lactate} \).

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 \( \text{\textalpha}-\text{lactate} \) concentrations accounted for 58% of the variation in venous blood pH and 77% of the variation in AG and SIG. Plasma \( \text{\textalpha} \)- and total lactate concentrations were slightly better correlated with SIG (\( r_s = -0.69 \); \( r_s = -0.78 \)) than to AG (\( r_s = 0.63 \); \( r_s = 0.74 \)).

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 \( A_{pot} \) when applying the simplified strong ion approach to acid-base balance to critically ill animals with hyperphosphatemia.

Key words: \( \text{\textalpha}-\text{lactate} \); Henderson-Hasselbalch model; Inorganic phosphorus; Strong ion difference.

Abbreviations:

| Abbreviation | Description |
|--------------|-------------|
| AG           | anion gap   |
| SIG          | strong ion gap |
| USI          | unidentified strong ions |

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 \( \text{\textalpha}-\text{lactate} \) 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 \( \text{\textalpha}-\text{lactate} \) concentrations\(^{3,4}\), but the recent discovery that increased plasma concentrations of \( \text{\textalpha}-\text{lactate} \) play an important role in the pathogenesis of metabolic (strong ion) acidosis 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 approach\(^{7}\) 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 \( \text{\textalpha}-\text{lactate} \). A nonvolatile buffer 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 (\( p\text{CO}_2 \), 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
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. 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,\textsuperscript{12,15} total protein-adjusted AG,\textsuperscript{8} SIG\textsuperscript{8}, and the related unmeasured anion (cXA) approach\textsuperscript{16} in quantifying the unmeasured strong anion and t-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\textsubscript{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 and L-lactate were determined from blood samples containing lithium heparin and potassium fluoride as a glycostatic agent. Concentrations of t- and l-lactate were determined photometrically by means of enzymatic methods using t- and l-lactate dehydrogenase.\textsuperscript{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,\textsuperscript{12,15} the total protein-adjusted AG,\textsuperscript{8} the SIG\textsuperscript{8}, and the related Figge et al unmeasured anion (cXA) approach.\textsuperscript{16} The AG, which is defined as the difference between unmeasured cations and unmeasured anions, was calculated in mEq/L as follows\textsuperscript{12,15}:

\[
AG = (\text{cNa}^+ + \text{cK}^+) - (\text{cCl}^- + \text{cHCO}_3^-).
\]

The calculated values for AG can be adjusted for changes in the albumin or total protein concentration.\textsuperscript{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,\textsuperscript{16,22,23} whereas studies in domestic animals have shown that globulin carries a net negative charge.\textsuperscript{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:\textsuperscript{8}

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

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\textsubscript{tot}, the experimentally determined value for the negative logarithm of dissociation constant of plasma nonvolatile weak acids (pK\textsubscript{a} = 7.08), and the following equation, whereby:\textsuperscript{8}

\[
SIG = A_{tot}/(1 + 10^{(7.08-\text{pH})}) - AG.
\]
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:

\[
\text{XA} = c\text{Na}^+ + c\text{K}^+ + c\text{Mg}^{2+} + c\text{Ca}^{2+} - c\text{Cl}^– - c\text{HCO}_3^– – (\text{protein charge}) – (\text{phosphorus charge}).
\]  

(6)

As the originally reported calculations for protein charge of human plasma\textsuperscript{16} are not applicable to bovine plasma, the charge of albumin and globulin was calculated according to Darrow and Hartmann\textsuperscript{25} as suggested by Constable\textsuperscript{11} with albumin and globulin expressed in g/L:

\[
\text{Albumin charge} = (0.141 \times [\text{albumin}]) \times (\text{pH} – 5.42),
\]  

(7)

\[
\text{Globulin charge} = (0.04 \times [\text{globulin}]) \times (\text{pH} – 5.58).
\]  

(8)

The charge of phosphorus was calculated as suggested by Figge et al\textsuperscript{16} with phosphorus concentration expressed in mmol/L:

\[
\text{Phosphorus charge} = [\text{phosphorus}] \times (0.309 \times \text{pH} – 0.469).
\]  

(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 follows:\textsuperscript{5}:

\[
\text{SID}_3 = c\text{Na}^+ + c\text{K}^+ – c\text{Cl}^–.
\]  

(10)

The measured strong ion difference obtained from seven strong ions\textsuperscript{25} (SID\textsubscript{3}, mEq/L) was calculated using the measured value for [Ca\textsuperscript{2+}]\textsuperscript{3} determined by ion-selective potentiometry and assigning a charge of +1.38 to magnesium assuming 69% dissociation\textsuperscript{16} and –1 to α-lactate and α-lactate assuming 100% dissociation such that:

\[
\text{SID}_3 = c\text{Na}^+ + c\text{K}^+ + c\text{Mg}^{2+} + c\text{Ca}^{2+} – c\text{Cl}^– – c(L - \text{lactate})
\]  

\[
– c(D - \text{D-lactate}).
\]  

(11)

The concentration of nonvolatile weak acids (A\textsubscript{tot}) in mmol/L in calf plasma was calculated from serum concentrations of total protein:\textsuperscript{6}

\[
A_{\text{tot}} = 0.343 \times c(\text{total protein}).
\]  

(12)

Calculated values for SIG were corrected for the measured concentrations of α- and γ-lactate and Ca\textsuperscript{2+} and Mg\textsuperscript{2+} to obtain the concentration of still unidentified strong ions (USI) in mEq/L for the calculation of the effective strong ion difference (SID\textsubscript{eff}):

\[
\text{USI} = \text{SIG} + c(D - \text{D-lactate}) + c(L - \text{lactate}) – c\text{Mg}^{2+} – c\text{Ca}^{2+},
\]  

(13)

\[
\text{SID}_{\text{eff}} = \text{SID}_3 + \text{USI} = \text{SID}_3 + \text{SIG}.
\]  

(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\textsubscript{25}/Q\textsubscript{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,\textsuperscript{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 (\( \Delta R^2 \)). 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.\textsuperscript{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.\textsuperscript{26} Moreover, the variance inflation factor for all predictors were <6; multicollinearity is typically considered a concern when the variance inflation factor exceeds 10.\textsuperscript{26}

Based on the nonlinear association between pH and HCO\textsubscript{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:

\[
\text{pH} = \log_{10}(a – \text{AG}) + b.
\]  

(15)

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

\[
\text{pH} = \log_{10}(\text{SID}_3 – \text{AG}) + (pK'_1 – \log_{10}(S \times \text{pCO}_2)).
\]  

(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:

\[
\text{pH} = \log_{10}(\text{SIG} + c) + d.
\]  

(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:

\[
A_{\text{tot}} (1 + 10^{\text{pH} - 7.08});
\]  

(18)

\[
\text{pH} = \log_{10}(\text{SIG} + \text{SIG} + A_{\text{tot}}/(1 + 10^{\text{pH} - 7.08}));
\]  

\[
+ (pK'_1 – \log_{10}(S \times \text{pCO}_2))
\]  

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

### Results

Availability of results of clinical biochemistry analysis allowed calculation of USI, SID\textsubscript{3}, SID\textsubscript{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:

\[
\text{pH} = \log_{10}(39.7 - \text{AG}) + 5.92. \tag{19}
\]

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 SID5 (38.7 mEq/L, Table 1).

The nonlinear equation relating pH to SIG was:

\[
\text{pH} = \log_{10}(\text{SIG} + 32.4) + 5.84. \tag{20}
\]

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 (\( A_{\text{tot}}, \) phosphorus concentration, \( p\text{CO}_3 \), and SID7) are depicted in Figure 3. A strong curvilinear association (\( r_s = 0.92, P < 0.001 \)) was observed between venous blood pH and SID7. The Spearman’s correlation coefficients between blood pH and serum phosphorus concentration, \( p\text{CO}_3 \), and \( A_{\text{tot}} \) were −0.53, 0.50, and −0.29, respectively.

To explore the importance of the SID7, \( A_{\text{tot}} \), and \( p\text{CO}_3 \) 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 \( \log_{10}\text{SID7} \), \( \log_{10}A_{\text{tot}} \), and \( \log_{10}p\text{CO}_3 \)) based on the mathematical relationships in equations 16, 17, and 18, \( c\text{HCO}_3^- \), and base excess as dependent variables (Table 3). The \( \log_{10}\text{SID7} \) and \( \log_{10}\text{SID3} \) had the highest explanatory power in the model for venous blood pH and base excess, whereas \( c\text{HCO}_3^- \) was best predicted by \( p\text{CO}_3 \). The final models explained 47, 71, and 63% of the variation in venous blood pH, actual \( c\text{HCO}_3^- \), 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 \( \text{D-lactate} \), \( \text{L-lactate} \), 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 \( p\text{CO}_2 \), were entered into a multivariable stepwise linear regression model (Table 4). The final model explained 75% of the variation in venous blood pH, \( \text{plasma D-lactate} \) 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 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. Scatterplots of calculated \( A_{\text{tot}} \) 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-

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. Scatterplots of calculated \( A_{\text{tot}} \) 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-
tion are associated with a decrease in plasma pH. This is compatible with clinical studies that reported a significant correlation between measures of clinical dehydration and the acid-base status of diarrheic calves. 

The calculated values for SID\textsubscript{7} (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 (SID\textsubscript{eff} + USI) was well correlated with venous blood pH with a non-linear relationship as previously reported, indicating that acidemia in neonatal diarrheic calves is predominantly the result of a strong ion acidosis.

The relative contribution of \(\log_{10}\) values for SID\textsubscript{7}, pCO\textsubscript{2}, and \(A_{tot}\) in determining the acid-base balance in diarrheic 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. 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 SID\textsubscript{7}, pCO\textsubscript{2}, and \(A_{tot}\).

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

| Variable                          | Median         | Q\textsubscript{25}/Q\textsubscript{75} | Reference Values |
|-----------------------------------|----------------|----------------------------------------|-----------------|
| Henderson-Hasselbalch model       |                |                                        |                 |
| Venous blood pH                   | 7.16           | 7.01/7.31                              | [7.35 to 7.50]\(^8\) |
| pCO\textsubscript{2} (mmHg)       | 47.1           | 37.3/55.4                              | [34 to 45]\(^8\) |
| HCO\textsubscript{3}^- (mmol/L)   | 15.8           | 9.8/26.4                               | [20 to 30]\(^8\) |
| Base Excess (mmol/L)              | -12.0          | -20.2/-0.6                             | [-3.5 to 3.5]\(^9\) |
| 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.            |
| Strong ion difference model       |                |                                        |                 |
| SID\textsubscript{3} (mEq/L)      | 38.7           | 34.5/42.9                              | [38.3 to 47.7]\(^8\) |
| SID\textsubscript{7} (mEq/L)      | 34.8           | 27.9/40.3                              | n.a.            |
| SID\textsubscript{eff} (mEq/L)    | 27.0           | 19.0/37.9                              | [37.3 to 51.5]\(^9\) |
| 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]\(^6\) |
| \(A^-\) (mEq/L)                  | 10.6           | 9.1/12.2                               | n.a.            |
| SIG (mEq/L)                       | -11.6          | -18.1/-2.1                             | [-3.0 to 3.0]\(^6\) |
| cXA (mEq/L)                       | 11.2           | 2.9/16.9                               | n.a.            |
| Clinical biochemistry analysis    |                |                                        |                 |
| 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]\(^8\) |
| Total protein (g/L)               | 57.3           | 50.2/64.7                              | [40.9 to 69.1]\(^9\) |
| Inorganic phosphorus (mmol/L)     | 3.1            | 2.5/4.0                                | [2.3 to 3.5]\(^9\) |
| Urea (mmol/L)                     | 13.4           | 7.6/22.4                               | [\(<10.8]\(^9\) |
| Creatinine (µmol/L)               | 148            | 105/301                                | [\(<159]\(^9\) |
| Electrolytes                      |                |                                        |                 |
| Sodium (mmol/L)                   | 135            | 130/142                                | [132 to 152]\(^8\) |
| Potassium (mmol/L)                | 4.9            | 4.3/6.0                                | [3.9 to 5.8]\(^8\) |
| Chloride (mmol/L)                 | 101            | 96/108                                 | [95 to 110]\(^8\) |
| Magnesium (mmol/L)                | 0.98           | 0.84/1.25                              | [0.74 to 1.10]\(^8\) |
| Ionized Calcium (mmol/L)          | 1.22           | 1.15/1.32                              | [1.2 to 1.6]\(^8\) |

\(n.a.,\) not available; pCO\textsubscript{2}, partial pressure of carbon dioxide; AG, anion gap; SID\textsubscript{3}, strong ion difference calculated from three strong cations and anions; SID\textsubscript{7}, strong ion difference calculated from seven strong cations and anions; SID\textsubscript{eff}, effective strong ion difference; USI, concentration of unidentified strong ions; \(A^-\), total net anion charge of nonvolatile weak acids; SIG, strong ion gap; cXA, unmeasured strong anion concentration according to Figge et al\(^16\).

### 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 ion concentration according to Figge et al\(^16\) (cXA), and venous blood pH, cHCO\textsubscript{3}^-, and base excess in 806 neonatal calves with diarrhea.

| Variable   | Venous Blood pH | cHCO\textsubscript{3}^- | Base Excess |
|------------|----------------|--------------------------|-------------|
| Anion gap  | -0.76**        | -0.72**                  | -0.75**     |
| Total      | -0.74**        | -0.72**                  | -0.75**     |
| protein-adjusted anion gap |             |                           |             |
| Strong ion gap | 0.83**        | 0.79**                   | 0.82**      |
| cXA        | -0.80**        | -0.80**                  | -0.82**     |

\(** P < .001.\)
Similarly to dehydrated adult cattle with right sided displacement of the abomasum or abomasal volvulus, 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, also play a role in the development of strong ion (metabolic) acidosis in dehydrated calves with diarrhea. A recent study 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 $d\Delta pH/\Delta$ strong ion difference ($+0.013 \text{ pH units}/(\text{mEq/L})$ in calf plasma. 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 $-0.023 (-1.8 \times 0.013)$. 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 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. 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 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.

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. 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. 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. This could be explained by the fact that a change in SID (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. However, in cases of a nonvolatile weak acid acidosis the resulting base excess value is additionally influenced by an increase in plasma concentration of nonvolatile 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.

Multivariable stepwise regression analysis indicated that the inorganic phosphorus concentration was the
most important predictor for an increase in AG as it was also reported for healthy calves.\textsuperscript{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,\textsuperscript{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.\textsuperscript{7,36}

The first method for calculating SIG was reported in 1995\textsuperscript{23} by using the cXA approach.\textsuperscript{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

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Order of Entry & Variable & $\Delta R^2$ & Model $R^2$ & Variance Inflation Factor \\
\hline
Venous blood pH as dependent variable & & & & \\
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 & - \\
Actual HCO$_3^-$ as dependent variable & & & & \\
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 \\
Base Excess as dependent variable & & & & \\
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 \\
\hline
\end{tabular}
\caption{Results of a stepwise linear regression analysis for predicting venous blood pH, $c\text{HCO}_3^-$, 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.}
\end{table}
groups of human albumin). Unfortunately, the 1995 calculations cannot be applied to bovine plasma because bovine globulins carry also a net anion charge, which is different from the situation in humans. 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. Although the same approach was reported to be inferior to SIG in predicting plasma \( \ell \)-lactate concentrations in adult cattle with abomasal volvulus, we found that \( \ell \times \) XA had the best correlation with \( \ell \)-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, \( \ell \times \) XA was still moderately correlated with phosphorus concentration which indicates inaccuracy 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 \( \ell \)-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, which suggests that \( \ell \)-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, our results demonstrate that the AG represents a clinically useful indicator of unmeasured strong anions such as \( \ell \)-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 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 | \( \Delta R^2 \) | Model \( R^2 \) | Coefficient | \( \pm SE \) | \( P \)-Value | Variance Inflation Factor |
|----------------|----------|----------------|----------------|-------------|----------|-------------|--------------------------|
| 1              | Constant | –              | –              | 7.34        | 0.22     | <.001       | –                        |
| 2              | \( \log_{10} \) L-lactate | 0.380       | 0.380         | –0.093      | 0.006    | <.001       | 1.35                     |
| 3              | \( \log_{10} \) Phosphorus | 0.176       | 0.556         | –0.648      | 0.023    | <.001       | 1.27                     |
| 4              | \( \log_{10} \) Chloride | 0.064       | 0.620         | –3.03       | 0.13     | <.001       | 3.51                     |
| 5              | \( \log_{10} \) Sodium | 0.111       | 0.731         | 3.02        | 0.17     | <.001       | 3.10                     |
| 6              | \( \log_{10} \) Calcium | 0.016       | 0.747         | 0.059       | 0.006    | <.001       | 1.16                     |

Constant – 7.70 0.05 <.001 –

### 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 | \( c \times \) XA Approach |
|-------------|-----------|----------------|----------------|---------------------------|
| Total lactate | 0.74**   | 0.78**         | –0.78**        | 0.82**                    |
| \( \ell \)-Lactate | 0.63**   | 0.68**         | –0.69**        | 0.75**                    |
| \( \ell \)-Lactate | 0.42**   | –0.03NS        | 0.05NS         | –1.14**                   |
| Phosphorus   | 0.71**   | 0.63**         | –0.65**        | 0.53**                    |
| Alummin      | 0.45**   | 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**                    |

\(* * P < .001; \text{NS} \) not significant.
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 hypophosphatemia.

### Footnotes

* Rapidlab® 865 blood gas analyzer, Bayer Vital GmbH, Fernwald, Germany
* Automatic Analyzer Hitachi 911, Roche Diagnostics, Indianapolis, IN
* IBM, New York
* SAS 9.3, SAS Inc., Cary, NC

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

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### Table 6

| Order of Entry | Variable          | An R² | Model R² | Coefficient | ±SE | P-value | Variance Inflation Factor |
|----------------|-------------------|-------|----------|-------------|-----|---------|---------------------------|
| **Anion gap**  |                   |       |          |             |     |         |                           |
| 1              | Phosphorus        | 0.447 | 0.447    | −9.70       | 1.03| <.001   |                           |
| 2              | D-Lactate         | 0.320 | 0.767    | 2.27        | 0.16| <.001   |                           |
| 3              | log₁₀Urea         | 0.030 | 0.797    | 0.00        | 0.00| <.001   |                           |
| 4              | L-Lactate         | 0.022 | 0.819    | 0.09        | 0.02| <.001   |                           |
| 5              | Globulin          | 0.009 | 0.828    | 0.18        | 0.04| <.001   |                           |
| 6              | Albumin           | 0.005 | 0.833    | 0.18        | 0.04| <.001   |                           |
| **Total protein-adjusted anion gap** |                   |       |          |             |     |         |                           |
| 1              | D-Lactate         | 0.407 | 0.407    | −1.88       | 0.51| <.001   |                           |
| 2              | Phosphorus        | 0.333 | 0.740    | 0.00        | 0.00| <.001   |                           |
| 3              | log₁₀Urea         | 0.033 | 0.773    | 0.00        | 0.00| <.001   |                           |
| 4              | L-Lactate         | 0.024 | 0.797    | 0.00        | 0.00| <.001   |                           |
| **Strong ion gap** |                   |       |          |             |     |         |                           |
| 1              | D-Lactate         | 0.419 | 0.419    | −1.17       | 0.03| <.001   |                           |
| 2              | Phosphorus        | 0.354 | 0.772    | −2.67       | 0.16| <.001   |                           |
| 3              | log₁₀Urea         | 0.034 | 0.806    | −7.78       | 0.61| <.001   |                           |
| 4              | L-Lactate         | 0.015 | 0.821    | −0.63       | 0.08| <.001   |                           |
| **cXA approach** |                   |       |          |             |     |         |                           |
| 1              | D-Lactate         | 0.511 | 0.511    | −10.10      | 0.55| <.001   |                           |
| 2              | log₁₀Urea         | 0.193 | 0.704    | 0.16        | 0.03| <.001   |                           |
| 3              | L-Lactate         | 0.060 | 0.764    | 0.02        | 0.06| <.001   |                           |
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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.