Calculation of variables describing plasma nonvolatile weak acids for use in the strong ion approach to acid-base balance in cattle

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Objective—To calculate values for the total concentration of nonvolatile weak acids (Atot) and the effective dissociation constant for nonvolatile weak acids (Ka) of bovine plasma and to determine the best method for quantifying the unmeasured strong anion concentration in bovine plasma.

Sample Population—Data sets from published and experimental studies.

Procedure—The simplified strong ion model was applied to published and experimentally determined values for pH, PCO₂, and strong ion difference (SID). Nonlinear regression was used to solve simultaneously for Atot and Ka. Four methods for quantifying the unmeasured strong anion concentration in plasma (anion gap, the Fencl base excess method [BEua], the Fige unmeasured anion method [XAI], and the strong ion gap [SIG]) were compared in 35 cattle with abomasal volvulus.

Results—For bovine plasma at 37°C, Atot was 25 mM/L, equivalent to 7.6 times the albumin concentration or 2.6 times the total protein concentration; Ka was 0.87 x 10⁻⁷, equivalent to pKa of 7.06. The Atot values were validated, using data sets from in vivo and in vitro studies. Plasma unmeasured strong anion concentration was most accurately predicted in critically ill cattle by calculating SIG from serum albumin (R², 0.66) or total protein concentration (R², 0.50), compared with BEua (R², 0.56); [XAI] (R², 0.50), and the anion gap (R², 0.41).


Changes in acid-base balance and plasma electrolyte concentrations frequently are evident in sick cattle as well as after a change in diet in healthy cattle. Circadian changes in blood pH and plasma electrolyte concentrations related to feeding also have been observed. From a clinical and nutritional perspective, it can be valuable to determine the mechanism for changes in acid-base status and plasma electrolyte concentrations in cattle. Two mechanistic acid-base models that are based on the strong ion approach have been developed to assess acid-base status: the strong ion model and the simplified strong ion model. Clinical application of these models to acid-base disturbances in domestic animals has been discussed.

The strong ion approach states that plasma pH is dependent on 3 independent variables: PCO₂; the difference between the charge of plasma strong cations (sodium, potassium, calcium, magnesium) and strong anions (chloride, lactate, sulfate, ketoacids, nonesterified fatty acids, and many others) termed the strong ion difference (SID); Appendix), in which strong cations and anions are fully dissociated at physiologic pH; and the total concentration of nonvolatile buffers (albumin, globulins, and inorganic phosphate) in plasma (Atot). Nonvolatile buffers are not fully dissociated at physiologic pH, which is in marked contrast to the complete dissociation of strong cations and anions. For a weak acid (HA)–conjugate base (A⁻) that is not fully dissociated, Atot is defined as follows: Atot = [HA] + [A⁻], because the net charge assigned to albumin, globulin, and phosphate in plasma is negative, and the conventional dissociation reaction is defined as follows: HA ⇌ H⁺ + A⁻. At equilibrium, an apparent weak acid dissociation constant (Ka) for nonvolatile buffers can be calculated as follows: Ka = [H⁺][A⁻]/[HA]. It should be mentioned that plasma pH is actively defended by changes in PCO₂ (respiratory system) and SID (urinary and gastrointestinal systems) and that acid-base status is not typically regulated by changes in Atot.

The strong ion approach requires accurate species-specific values for Atot and Ka. Values for Atot (14.9 or 15.0 mM/L) and Ka (2.1 x 10⁻⁷ or 2.2 x 10⁻⁷) have been experimentally determined for equine plasma, but other values for Atot (24.1 mM/L) and Ka (1.0 x 10⁻⁷) have been calculated for human plasma. Results of a study in milk-fed calves suggested that Atot is 18.3 mM/L, and Ka is 6.8 x 10⁻⁷, although the calculated value for Ka was greater than the expected value of 1.0 x 10⁻⁷. Accordingly, the first objective of the study reported here was to calculate accurate values for Atot and Ka for bovine plasma. This was accomplished by use of published data from 2 in vitro studies. Published pKa values for amino acids in bovine plasma and albumin, results of an in vitro study of CO₂ tonometry of bovine plasma, calculated Atot and Ka values then were validated, using data obtained from an in vivo study in calves with experimentally induced respiratory and metabolic acidosis and an in vitro study of CO₂ tonometry of bovine serum.

The second objective was to compare 4 methods for quantifying the unmeasured strong anion concentration in bovine plasma. These 4 methods were the traditional anion gap approach, the Fencl base-excess method,
(BFun)\textsuperscript{22,23} the Figge unmeasured anion method ([IA]),\textsuperscript{24} and the strong ion gap (SIG) method.\textsuperscript{21}

Materials and Methods
Calculation of $A_{\text{tot}}$ and $K_a$ values for bovine plasma—
Data were obtained from 2 in vitro studies.\textsuperscript{17,18} The former data set involved titration of bovine plasma at 38 C with hydrochloric acid and sodium carbonate.\textsuperscript{11} The simplified strong ion electroneutrality equation\textsuperscript{a} was applied as follows (equation 1):

$$S \cdot P_{\text{CO}_2} \cdot 10^{[\text{pH}]} = \text{SID} – (A_{\text{tot}}/1 + 10^{[\text{pH}]}).$$

where $S$ is plasma solubility of $\text{CO}_2$, $P_{\text{CO}_2}$ is the negative logarithm (base 10) of the apparent dissociation constant for carbonic acid, and $K_a$ is the negative logarithm (base 10) of $K_a$. The algebraic form of the simplified strong ion equation\textsuperscript{a} used in equation 1 was selected because of the fact it provided the closest confidence intervals for estimates of $A_{\text{tot}}$ and $K_a$. Equation 1 was applied to the reported values for pH, $P_{\text{CO}_2}$, and SID, the latter variable being calculated from the reported base-excess value for plasma, using the following equation: $\text{SID} = \text{base excess} + 44$. The addition of 44 to the base-excess value was required, because the normal base-excess value in bovine plasma is approximately +2 (when pH is 7.43, $P_{\text{CO}_2}$ is 43 mm Hg, and $\text{HCO}_3^-$ is 26.4 mm/L), indicating that $\text{SID} = 41.7 + 2$, which is approximately 44. $P_{\text{CO}_2}$. The calculated value for $\text{SID}$ was extrapolated from the value for human plasma, where base excess is defined as 0 when pH is 7.40, $P_{\text{CO}_2}$ is 40 mm Hg, and $\text{HCO}_3^-$ is 24 mm/L.\textsuperscript{3,4} Although it is acknowledged that the value of 44 mEq/L for the SID of bovine plasma is approximate, the estimate agreed with that obtained by summing the charge attributed to strong cations and anions in bovine plasma (approximately 44 mEq/L; Appendix).

Data analysis was restricted to base-excess values between –15 and +15 mEq/L, because residual plots developed during nonlinear regression analysis indicated deviation of fitted from actual values outside this range, presumably because the hypertonic solutions used for strong ion titration increased ionic strength, thereby altering the effective values for $K_a$ and $K_a$.\textsuperscript{6} Titration was accomplished at 38 C, therefore, temperature-adjusted values for $S$ (0.0301 mm/L/mm Hg)\textsuperscript{5} and $P_{\text{CO}_2}$ (6.120) were used.\textsuperscript{6} Nonlinear regression analysis was used to solve simultaneously for $A_{\text{tot}}$ and $K_a$, using reported or derived values for pH, $P_{\text{CO}_2}$, and SID, known values for $S$ and $P_{\text{CO}_2}$, equation 1, and the Marquardt expansion algorithm.\textsuperscript{6} The 6-factor simplified strong ion model\textsuperscript{6} was used for nonlinear regression analysis instead of the 8-factor strong ion model,\textsuperscript{1} because reducing the number of variables in the model leads to more precise estimates.\textsuperscript{11} Initial estimates for $A_{\text{tot}}$ of 5 to 30 mEq/L in increments of 5 mEq/L and for $K_a$ of 0.5 $\times$ $10^{[3]}$ to $3.0 \times 10^{[2]}$ in increments of 0.5 $\times 10^{[2]}$ were used. The calculated value for $K_a$ (–log$_e$ $K_a$ obtained at 38 C) was corrected to 37 C, using the van ‘t Hoff equation as follows (equation 2):

$$K_a = \text{p}K_a - \text{pK}_\text{a}(\sqrt{\text{AT} \times 2.303R}/1/T).$$

where $\text{AT}$ is 6.940 cal/mol, R (the gas constant) is 1.9871 cal/K/mol, T is the temperature (in degrees Kelvin).\textsuperscript{31} This provided the following equation:

$$\text{p}K_a = \text{p}K_a (\text{37 C}) - 0.016.$$

The second data set involved titration of combined bovine and ovine plasma, using hydrochloric acid and sodium carbonate in addition to hemoglobin concentrations of 5, 10, and 15 g/dl at 38 C.\textsuperscript{14} Data analysis was completed for the 3 hemoglobin concentrations in a manner similar to that described previously for plasma. Linear regression analysis of the calculated $A_{\text{tot}}$ or $K_a$ values against hemoglobin concentration (5, 10, and 15 g/dl) was performed, and the intercept value (hemoglobin concentration of 0 g/dl) was used as the estimated value for $A_{\text{tot}}$ and $K_a$. Plasma extrapolation method was required, because hemoglobin has $A_{\text{tot}}$ and $K_a$ values that differ from those of plasma. The calculated value for $K_a$ obtained at 38 C was corrected to a value for 37 C, using equation 2 as described previously.

Calculation of $K_a$ for bovine albumin, using $pK_a$ values for dissociable amino acid groups—Tanford et al\textsuperscript{19} identified 215 dissociable groups on bovine albumin that could be categorized into 7 groups that had an effective $K_a$ at 25 C as follows: 1) a-carboxy terminus group, $pK_a = 3.75$; 99 $\beta$- and $\gamma$- groups, $pK_a = 4.02$; 16 imidazole groups, $pK_a = 6.9$; 1 $\alpha$-amino group, $pK_a = 7.75$; 57 $\epsilon$-amino groups, $pK_a = 9.88$; 19 phenolic groups, $pK_a = 10.35$; and 22 guanidine groups, $pK_a > 12$. An apparent $pK_a$ for bovine albumin was calculated from this data, using the weighted mean average of the 215 dissociable groups and assuming the $pK_a$ of guanidine was 12.5.

Calculation of $A_{\text{tot}}$ and $K_a$ values, using in vitro CO$_2$ tonometry—Jugular venous blood was collected anaerobically from 6 healthy adult cattle (3 females, 3 males) into tubes containing heparin sodium. Samples were centrifuged, and plasma was harvested. Plasma samples were equilibrated at 37 C for 20 minutes with a gas that contained CO$_2$ by use of a tonometer.\textsuperscript{21} Varying mixtures of 100% O$_2$ and 100% CO$_2$ were used to provide a wide range of $P_{\text{CO}_2}$. The pH and $P_{\text{CO}_2}$ of the tonometered plasma samples were determined at 37 C, using a pH-blood gas analyzer.\textsuperscript{22} Plasma concentrations of sodium, potassium, and chloride (ion-selective electrode potentiometry), albumin (brom cresol green method), total protein (biuret method), and phosphorus (ammonium molybdate method) were determined, using an automatic analyzer. Plasma lactate concentration was determined, using a spectrophotometric method and lactate dehydrogenase.\textsuperscript{6} Measured SID was calculated as follows:

$$\text{SID} = [\text{Na}^+] + [K^+] – [\text{Cl}^–] – [\text{lactate}].$$

where all values were in mEq/L. Data analysis, using nonlinear regression, equation 1, and the measured values for pH, SID, and $P_{\text{CO}_2}$, was completed as described previously to provide estimates of $A_{\text{tot}}$ and $K_a$ values in plasma for each of the cattle. Because titration was accomplished at 37 C, temperature-adjusted values for $S$ (ie, [0.0307 mm/L/mm Hg])\textsuperscript{5} and $P_{\text{CO}_2}$ (ie, 6.129) were used.\textsuperscript{6}

Validation of calculated $A_{\text{tot}}$ and $K_a$ values, using data from an in vivo study—The calculated $A_{\text{tot}}$ and $K_a$ values were applied to data obtained from an in vivo study that involved 8 calves with acute mixed respiratory and metabolic acidosis.\textsuperscript{1} Plasma pH was calculated by use of the simplified strong ion equation\textsuperscript{1} as follows (equation 3):

$$\text{pH} = \log_{10}(2S/\text{SID}) + [K_a \cdot \text{SID}^+] + ([K_a \cdot \text{SID} + [K_a \cdot \text{SID} + [K_a \cdot \text{Atot}^+] – 4[K_a \cdot \text{SID} + \text{Atot}^+]])$$

where $K_a$ is the apparent dissociation constant for carbonic acid. Plasma pH was calculated from reported values for SID and $P_{\text{CO}_2}$ as well as total protein or albumin concentrations. Measured SID was calculated as follows:

$$\text{SID} = [\text{Na}^+] + [K^+] – [\text{Cl}^–] – [\text{lactate}]$$

where sodium, potassium, and chloride concentrations were determined by use of ion-selective electrode potentiometry,\textsuperscript{23} and lactate concentration was determined by the lactate dehydrogenase method.\textsuperscript{6} Value for $A_{\text{tot}}$ was calculated from
Validation of calculated A\text{tot} and K\text{e} values, using data from in vitro studies—Calculated A\text{tot} and K\text{e} values were applied to data obtained from a report of an in vitro study that involved CO2 tonometry of bovine serum at 38 C. Plasma pH was calculated, using equation 3, reported values for PCO2, and calculated values for A\text{tot} and K\text{e}. The value for SID was assumed to be 44 mEq/L. The value for pH\text{calc} was regressed against the value for pH\text{meas}.

Quantifying unmeasured strong anion concentration in plasma—A clinically important problem in sick cattle is identifying and quantifying strong anions in plasma that are not routinely measured such as lactate, β-hydroxybutyrate, acetocetate, and anions associated with uremia. Unmeasured strong anions traditionally have been quantified by calculating the anion gap, using the following equation:

\[ \text{Anion gap} = ([\text{Na}^+] + [\text{K}^+] - ([\text{Cl}^-] + [\text{HCO}_3^-]) \]

The concentration of HCO3\text{−} was calculated as follows:

\[ [\text{HCO}_3^-] = 5 \times \text{PCO}_2 \times 10^{(pH - pK'_1)} \]

This method requires measurement of 5 factors (pH, PCO2, and concentrations of sodium, potassium, and chloride) or 4 factors (total CO2 and concentrations of sodium, potassium, and chloride) and has been applied to acid-base disturbances in cattle.

A second method for quantifying unmeasured strong anion concentration\textsuperscript{22,23} was developed by Fencl. That method compartmentalizes base excess (BE) in units of milliequivalents per liter into various components. These include BE changes in plasma free water (BE\text{fw}), which is calculated by use of the following equation:

\[ \text{BE}_{\text{fw}} = 0.30 \times ([\text{Na}^+] - 144) \]

where the reference value for mean plasma sodium concentration (144 mEq/L) is provided, and BE changes in plasma chloride concentration (BE\text{Cl}), which is calculated by use of the following equation:

\[ \text{BE}_{\text{Cl}} = 104 - ([\text{Cl}^-] \times 144/[\text{Na}^+]) \]

where the reference values of the mean plasma concentrations of sodium (144 mEq/L) and chloride (104 mEq/L) are provided. It also includes BE changes in plasma protein concentration [BE\text{albumin}], which is calculated by use of the following equation:

\[ \text{BE}_{\text{albumin}} = 0.28 \times 10 \times ([3.3 - [\text{albumin}]) \]

where the reference value of the net charge at pH 7.43 (ie, 0.28 mEq/g of bovine albumin)\textsuperscript{24} and the reference value for mean plasma albumin concentration (3.3 g/dl) is provided, or as BE changes in plasma total protein concentration [BE\text{total protein}], which is calculated by use of the following equation:

\[ \text{BE}_{\text{total protein}} = 0.17 \times 10 \times (7.0 - [\text{total protein}]) \]

where the value of 0.17 mEq/g of bovine plasma protein is provided for the net charge at reference mean pH,\textsuperscript{25} and the reference value for mean plasma protein concentration (7.0 g/dl) also is provided.

Estimates for the unmeasured strong anion concentration, using plasma concentrations of albumin, were calculated by use of the following equation:

\[ \text{BE}_{\text{ua-albumin}} = \text{BE} - (\text{BE}_{\text{fw}} + \text{BE}_{\text{Cl}} + \text{BE}_{\text{albumin}}) \]

Estimates for the unmeasured strong anion concentration, using plasma concentrations of total protein, also were calculated, using the following equation:

\[ \text{BE}_{\text{ua-total protein}} = \text{BE} - (\text{BE}_{\text{fw}} + \text{BE}_{\text{Cl}} + \text{BE}_{\text{total protein}}) \]

For both of these equations, base excess was calculated as standard base excess (also termed in vivo base excess or the base excess of extracellular fluid) from the pH and PCO2 values, using the van Slyke equation and assuming that blood hemoglobin concentration is 5 g/dl.\textsuperscript{26} The Fencl method requires measurement of 6 factors (pH, PCO2, and concentrations of sodium, chloride, albumin or total protein, and phosphate) and has been applied to acid-base disturbances in cattle.\textsuperscript{27,28}

A third method for quantifying the unmeasured strong ion concentration was proposed by Figge et al.\textsuperscript{9} They developed a general equation for quantifying the unmeasured anion concentration in plasma as follows:

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

This method required measurement of 9 factors (pH, PCO2, and concentrations of sodium, potassium, calcium, magnesium, chloride, albumin, and phosphate) and assigned all protein charges in plasma to albumin.\textsuperscript{9} Because bovine globulin carries a net negative charge,\textsuperscript{19} which is contrary to the situation for human globulin,\textsuperscript{17} the method was adapted for bovine plasma, using the charge assigned to bovine albumin and globulin by Darrow and Hartman.\textsuperscript{18} For bovine albumin, the following equation was used:

\[ \text{Albumin charge} = (1.41 \times [\text{albumin}]) \times (\text{pH} - 5.42) \]

with albumin concentration reported in grams per deciliter. For bovine globulin, the following equation was used:

\[ \text{Globulin charge} = (0.40 \times ([\text{total protein} - \text{albumin}]) \times (\text{pH} - 5.58) \]

with albumin and total protein concentrations reported in grams per deciliter.

Charge was assigned to phosphate as suggested by Figge et al,\textsuperscript{9} using the following equation:

\[ \text{Phosphate charge} = (0.099 \times [\text{phosphate}]) \times (\text{pH} - 1.52) \]

with phosphate concentration reported in milligrams per deciliter.

The SIG was the fourth method for estimating the unmeasured strong anion concentration. Kellum et al\textsuperscript{31} first defined the SIG as the difference between the charge assigned to unmeasured strong cations and unmeasured strong anions and was calculated using the [XA] approach. An alternative method for calculating SIG was developed by Constable et al\textsuperscript{32} from the simplified strong ion model,\textsuperscript{1} requiring measurement of 6 factors (pH, PCO2, and concentrations of sodium, potassium, chloride, and total protein or albumin) and calculation of the anion gap, using the following equation:

\[ \text{SIG} = A_{\text{tot}}/[1 + 10^{-(pH - 5.42)}] - \text{anion gap} \]

The 4 methods for quantifying unmeasured strong anions in plasma (anion gap, BE\text{ua} [XA], and SIG) were compared, using data obtained from cows with abomasal volvulus.\textsuperscript{15} The relationship between anion gap, BE\text{ua} [XA], and SIG and plasma l-lactate concentration was determined, using linear regression.
Sensitivity of plasma pH to changes in SID', Pco2, and Atot—Sensitivity of the dependent variable (plasma pH) to changes in the 8 factors of the Stewart strong ion model (SID', Pco2, Atot, S, K1', K2, K3, the apparent dissociation constant for bicarbonate [K3'], and the apparent dissociation constant for water [Kw']) and the 6 factors of the simplified strong ion model (SID', Pco2, Atot, S, K1', K2) were conveyed by a spider plot, which graphically depicted the relationship between the dependent variable and percentage change in 1 factor while the remaining factors were held constant at typical values. Using equation 3 (ie, the simplified strong ion model), the derivatives of pH with respect to the 3 independent factors SID', Pco2, and Atot of the strong ion approach were calculated to provide an index of the sensitivity of pH to changes in each of the independent factors. For SID', the following equation was used:

$$\Delta \text{pH} / \Delta \text{SID}' = (1 + 10^{(pK'_{a} - p\text{SID}' + p\text{co}}} / L) \times [\text{SID}' \times 10^{(pK'_{a} - p\text{SID}')}] - (\text{SID}' \times 10^{(pK'_{a} - p\text{SID}')})$$

For Pco2, the following equation was used:

$$\Delta \text{pH} / \Delta \text{Pco2} = (2.303[\text{S} \times \text{Pco2} \times 10^{(pK'_{a} - p\text{co2})}] + [\text{Atot} \times 10^{(pK'_{a} - p\text{co2})}]) - (\text{SID}' \times 10^{(pK'_{a} - p\text{co2})})$$

For Atot, the following equation was used:

$$\Delta \text{pH} / \Delta \text{Atot} = \text{pK'_{a} / S} \times [2.303(\text{S} \times \text{Pco2} \times 10^{(pK'_{a} - p\text{co2})}] + \text{Atot} \times 10^{(pK'_{a} - p\text{co2})})] - (\text{SID}' \times 10^{(pK'_{a} - p\text{co2})})$$

These 3 equations were solved by use of typical physiologic values for bovine plasma harvested from blood samples obtained from the jugular vein in which pH is 7.43, SID' is 44 mEq/L, Pco2 is 43 mm Hg, S is (0.0307 mML/mmm Hg, and pK' for bovine albumin is 6.129 as well as by the use of calculated values for Atot and pK' for bovine albumin.

Results

Calculation of Atot and Ks values for bovine plasma—Calculation of the first data set containing 7 data points from strong ion titration at 38 C of plasma from red-pied cows' yielded values for Atot of 25.0 mML/L and K1 of 0.90 X 10^-7. The value for K1 at 37 C was calculated using the van 't Hoff equation, and yielded 0.87 X 10^-7; pK' was 7.06. Assuming reference values for total protein (7.0 g/dl) and albumin (3.3 g/dl) concentrations, the value for Atot is equal to 3.6 times the total protein concentration or 7.6 times the albumin concentration.

Analysis of the second data set containing 42 data points from strong ion titration at 38 C of plasma from 8 calves and 2 sheep' yielded values for Atot of 29.9 mML/L and K1 of 0.17 X 10^-7. The value for K1 at 37 C was calculated using the van 't Hoff equation, and yielded 0.13 X 10^-7; pK' was 6.99. Because this data set involved plasma for 2 species and used inaccurate methods for measuring hemoglobin concentration (a hemoglobinometer) and total protein concentration (copper sulfate method), the calculated Atot and Ks values, although similar, were suspected to be not as accurate as those calculated by use of plasma from red-pied cows.17

Calculation of Ks value for bovine albumin, using pKs values for dissociable amino acid groups—Analysis of data obtained by titration of bovine albumin' was conducted by use of the following equation:

$$pK_{s} = [(1 \times 3.75) + (99 \times 4.02) + (16 \times 6.9) + (1 \times 7.75) + (57 \times 9.8) + (19 \times 10.33) + (22 \times 12.5)] / 215$$

This resulted in an apparent pKs for albumin of 7.210 at 25 C. Using equation 2, the estimated pKs value at 37 C was calculated and found to be 7.01. This approximated the value calculated previously for plasma.

Calculation of Atot and Ks values, using in vitro CO2 tonometry—Analysis of tonometry data for plasma from 6 healthy adult cattle provided values for Atot (mean ± SD, 22.9 ± 9.0 mML/L, range, 12.1 to 32.9 mML/L) and K1 (mean, 0.9 ± 1.2 X 10^-7; range, 0.1 X 10^-7 to 5.9 X 10^-7). Measured SID' ranged from 38.3 to 41.7 mEq/L (mean, 40.5 ± 1.2 mEq/L), plasma total protein concentration ranged from 2.9 to 4.4 g/dl (mean, 3.7 ± 0.5 g/dl), and plasma albumin concentration ranged from 2.4 to 3.1 g/dl. Seven to 9 plasma samples from each animal were examined by use of tonometry. The pH ranged from 6.98 to 7.90, and Pco2 ranged from 15 to 175 mm Hg. This data set provided much larger variability in the estimate for Atot and K1 than those calculated by use of plasma from red-pied cows. Thus, although the mean estimates were similar, the Atot and Ks values obtained from the data set reported by Lebeda and Bouda17 were used for validation.

Validation of calculated Atot and Ks values, using data from in vivo studies—Calculated values of Atot (7.6 times the albumin concentration) and Ks (0.87 X 10^-7) for bovine plasma were applied to data obtained from an in vivo study' that involved 8 calves with acute mixed respiratory and metabolic acidosis. For the in vivo validation data set, pH ranged from 6.91 to 7.49, measured SID' ranged from 28.4 to 58.3 mEq/L, Pco2 ranged from 38 to 195 mm Hg, and albumin concentration ranged from 2.4 to 3.1 g/dl. When the calculated values for Atot and Ks derived from the data set of Lebeda and Bouda17 were used, a good-to-excellent correlation between pHcalc and pHmeas was observed for the 8 calves (mean R², 0.92; range, 0.79 to 0.99; Fig 1), and the regression equation relating pHcalc to pHmeas was not significantly different from the line of identity, as determined by use of the following equation:

$$\text{pH}_{\text{calc}} = ([0.95 ± 0.14] \times \text{pH}_{\text{meas}}) - (0.41 ± 0.02)$$

where the values indicated represented the mean ± SD.
for the estimate. Mean difference between $pH_{\text{calc}}$ and $pH_{\text{meas}}$ was $0.02 \pm 0.06$.

Validation of calculated $A_{\text{tot}}$ and $K_a$ values, using data from in vitro studies—Calculated $A_{\text{tot}}$ and $K_a$ values were applied to data obtained from a published in vitro study that involved CO$_2$ tonometry of bovine serum at 38°C. For the in vitro data set used for validation, pH ranged from 6.33 to 7.74, and Pco$_2$ ranged from 23 to 710 mm Hg. When the calculated values for $A_{\text{tot}}$ and $K_a$ were used, an excellent correlation between $pH_{\text{calc}}$ and $pH_{\text{meas}}$ was observed ($R^2, 0.992$; Fig 1), but the regression equation relating $pH_{\text{calc}}$ to $pH_{\text{meas}}$ differed slightly from the line of identity, as determined by use of the following equation:

$$pH_{\text{calc}} = ([0.92 \pm 0.03] \times pH_{\text{meas}}) + (0.59 \pm 0.20)$$

$$\text{where the values indicated represented the mean } \pm \text{ SE for the estimate, because linear regression analysis was performed only on 1 data set. Mean difference between } pH_{\text{calc}} \text{ and } pH_{\text{meas}} \text{ was } 0.009 \pm 0.051.$$

Quantifying unmeasured strong anion concentration in plasma—Blood gas values and results of serum biochemical analysis were available for 41 critically ill cattle with abomasal volvulus. The aforementioned equation was revised for albumin concentration, resulting in the following equation:

$$\text{SIG}_{\text{albumin}} = (7.6 \times \text{albumin}) / [1 + 10^{(7.06 – pH)}] – \text{anion gap}$$

The aforementioned equation also was revised for total protein concentration, resulting in the following equation:

$$\text{SIG}_{\text{total protein}} = (3.6 \times \text{total protein}) / [1 + 10^{(7.06 – pH)}] – \text{anion gap}$$

Examination of residual plots from all 6 linear regression equations identified data from 6 cows as statistical outliers, which were attributed to laboratory error or an increase in plasma strong anions other than chloride or l-lactate. Therefore, analysis was conducted on data from only 35 cows with abomasal volvulus.

Of the 4 methods for quantifying the unmeasured strong anion concentration, the $\text{SIG}_{\text{albumin}}$ equation provided the best fit for the data, as determined on the basis of $R^2$ values for the linear regression models (Fig 2; Table 1). This indicated that $\text{SIG}_{\text{albumin}}$ provided the most accurate method for predicting unmeasured strong anion concentration (plasma lactate concentration) in cattle with abomasal volvulus.

Sensitivity of plasma pH to changes in SID $^+$, Pco$_2$, and $A_{\text{tot}}$—Analysis of the spider plot revealed that

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**Table 1—Linear regression equations for 4 methods of quantifying the unmeasured strong anion concentration in plasma obtained from 35 cows with abomasal volvulus**

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of factors</th>
<th>Linear regression equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIG</td>
<td>6</td>
<td>$\text{SIG}_{\text{albumin}} = (0.05 \pm 0.13) \times \text{lactate} + (2.8 \pm 0.8)^*$</td>
<td>0.66</td>
</tr>
<tr>
<td>SIG</td>
<td>6</td>
<td>$\text{SIG}_{\text{total protein}} = (0.94 \pm 0.14) \times \text{lactate} + (3.9 \pm 0.8)^*$</td>
<td>0.60</td>
</tr>
<tr>
<td>BE$_{ua}$</td>
<td>6</td>
<td>$\text{BE}_{\text{ua-albumin}} = (1.04 \pm 0.16) \times \text{lactate} + (1.7 \pm 1.0)$</td>
<td>0.56</td>
</tr>
<tr>
<td>BE$_{ua}$</td>
<td>6</td>
<td>$\text{BE}_{\text{ua-total protein}} = (1.04 \pm 0.16) \times \text{lactate} + (2.1 \pm 1.0)^*$</td>
<td>0.55</td>
</tr>
<tr>
<td>[X]$A]$</td>
<td>9</td>
<td>$\text{[X]}A] = (0.76 \pm 0.13) \times \text{lactate} + (10.3 \pm 0.8)^*$</td>
<td>0.55</td>
</tr>
<tr>
<td>Anion gap</td>
<td>5</td>
<td>Anion gap = (0.87 \pm 0.18) \times \text{lactate} + (16.3 \pm 1.1)^*$</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*Value for the intercept differs significantly ($P < 0.05$) from 0.

The 4 methods are strong ion gap (SIG), which is based on albumin or total protein concentration; the Fencl base-excess method (BE$_{ua}$), which is based on albumin or total protein concentration; the Figge unmeasured anion method ([X]$A]$); and the anion gap. Values represent mean $\pm$ SE for the estimate.
plasma pH was most sensitive to changes in SID' (Fig 3). Furthermore, plasma pH was more sensitive to changes in $A_{tot}$ than to changes in $K_a$. The tangent to each line in the spider plot reflects the sensitivity of bovine plasma pH to that factor, whereby at the reference value for pH in samples obtained from the jugular vein (ie, pH 7.43), the following equation was used for SID':

$$\Delta \text{pH}/\Delta \text{SID}' = +0.014/(\text{mEq/L})$$

At venous pH of 7.43, the following equation was used for $PCO_2$:

$$\Delta \text{pH}/\Delta PCO_2 = -0.008/\text{mm Hg}$$

At venous pH of 7.43, the following equation was used for $A_{tot}$:

$$\Delta \text{pH}/\Delta A_{tot} = -0.0096/(\text{mM/L})$$

$$= -0.035/(\text{g of total protein/dl})$$

Interestingly, values for $K_3'$ or $K_w'$ did not alter pH, indicating that neither factor influenced plasma pH.

![Spider plot revealing the dependence of plasma pH on changes in the 3 independent variables (strong ion difference [SID'], $PCO_2$, and total concentration of nonvolatile weak acids [$A_{tot}$]) and 5 constant variables (plasma solubility of $CO_2$ (S), the apparent dissociation constant for carbonic acid [$K_1$], effective dissociation constant for nonvolatile weak acids [$K_a$], the apparent dissociation constant for bicarbonate [$K_3$], and the apparent dissociation constant for water [$K_w$]) of the Stewart strong ion model$^6$ and the 3 independent variables (SID', $PCO_2$, $A_{tot}$) and 3 constants ($S$, $K_1$, $K_a$) of the simplified strong ion model.$^6$ The spider plot was obtained by systematically varying 1 input variable while holding the remaining input variables at their reference values for bovine plasma. Values for input variables were as follows: SID', 44 mEq/L (open triangle); $PCO_2$, 43 mm Hg (open square); $A_{tot}$, 25 mM/L (solid circle); $S$, 0.307 mEq/L/mM Hg (open square); $K_1$, 7.43 X 10$^{-7}$ (open square); $K_a$, 6.0 X 10$^{-4}$ (solid square); $K_3$, 6.0 X 10$^{-4}$ (open circle); and $K_w$, 4.4 X 10$^{-14}$ (solid triangle). Notice that SID', $PCO_2$, and $A_{tot}$ have the same symbol, which is attributable to the fact that the influence of $S$ and $K_1$ on plasma pH cannot be separated from that of $PCO_2$ because the 3 factors always appear as 1 expression. Large changes in 2 factors ($K_a$ and $K_3'$) did not change plasma pH, indicating that the Stewart strong ion model has too many variables.

### Discussion

Analysis of the findings of the study reported here indicated that the $A_{tot}$ and $K_a$ values for bovine plasma were similar to those for human plasma but differed from those for equine plasma. This confirms findings$^{35,36}$ and suspicions$^{37-39}$ that species-specific $A_{tot}$ and $K_a$ values are required. The finding that the $A_{tot}$ and $K_a$ values for bovine and human plasma are similar is consistent with observations that the pH-$PCO_2$ coordinates for the base-excess curve of bovine plasma are parallel to those for human plasma.$^{17,20,21}$

There are 2 main reasons to apply the strong ion approach to acid-base disturbances in cattle: to identify the mechanism for a change in acid-base balance and thereby focus treatment on the inciting cause and to quantify unmeasured strong anion concentration in plasma. The strong ion approach should never be used to calculate plasma pH, because pH can be measured much more accurately than it can be calculated, as evidenced by the large degree of scatter around the line of identity (Fig 1). Sensitivity analysis further emphasizes the accuracy with which SID' must be measured to calculate plasma pH, because a change of 1 mEq/L in SID' at reference value pH (ie, 7.43) will change pH by 0.014 (Fig 3). Inaccurate measurement of SID' was the most likely reason for the large scatter in the calculated pH values for the identity line, although some of the scatter also may have been attributable to calculating the $A_{tot}$ value from the plasma albumin concentration, because the plasma phosphorus concentration is higher in calves than in adult cattle.

The major inaccuracy with the strong ion approach is obtaining an accurate value for SID'. Determination of SID' requires identification and measurement of all strong ions in plasma, which is an impossible task because unidentified strong anions such as lactate, β-hydroxybutyrate, acetoacetate, non-esterified fatty acids, acetate, and sulfate may be increased in sick cattle (Appendix). Despite this shortcoming, an estimate of SID' can be obtained by determining the plasma concentration of 4 strong ions (sodium, potassium, chloride, and lactate), whereby SID' = ($[Na]$ + [$K$] - [$Cl$] - [lactate]). Additional strong cations (calcium and magnesium) could be included in the calculation of SID', although it remains to be determined whether their inclusion provides useful additional information when estimating a value for SID'. It should be recognized that regardless of which strong cations and anions are included in the calculation of SID', these calculations provide only an estimate for SID', because differing analytical methods for determination of concentrations of sodium, potassium, chloride, and lactate result in quantitatively large inter-laboratory differences in SID'; each method assumes that the sum of the unmeasured strong cations equals the sum of the unmeasured strong anions; unmeasured strong ions may become quantitatively important in specific pathologic states such as β-hydroxybutyrate and acetoacetate in ketoacidosis and sulfate in uremia; and each individual measurement is subject to error, creating a much larger cumulative error in SID'. Therefore, the terms measured SID' or estimated SID' should be used whenever the strong ion approach is
used clinically or experimentally. Use of the term SID, instead of measured SID, is misleading in that it implies that all strong anions and cations have been accurately measured, which is impossible. An example of the need for an accurate estimate of SID was provided in a study of acid-base disturbances in diarrheic calves, whereby SID was incorrectly calculated by use of the following equation: SID = [Na] + [K] – [Cl]. This produced the inevitable conclusion by the authors that measurement of SID as a means of evaluating the acid-base status of diarrheic calves may be so misleading that it could be dangerous. This statement is certainly true when SID is calculated only from the sodium, potassium, and chloride concentrations in animals with increased plasma concentrations of strong anions such as lactate, acetate, β-hydroxybutyrate, acetoacetate, nonesterified fatty acids, and sulfate. In other words, unless an accurate estimate of SID is available, the strong ion approach will have limited clinical application in the diagnosis and treatment of acid-base disorders in sick cattle, with the 1 exception being that the strong ion approach can quantify the difference between unmeasured strong cation and anion concentrations in plasma.

An alternative method for estimating the SID value of bovine plasma makes use of the following equation: SIDbovine = SIDhuman + BEplasma = 41.7 + BEplasma. This method is only valid when the concentrations of plasma nonvolatile buffers (albumin, globulin, and inorganic phosphate) are within reference ranges, because for these circumstances, the change in SID from its reference value is equivalent to the change in base excess from its reference value. Base excess can be calculated, using the Sigggaard-Andersen empirical equation derived from a nomogram, which simplifies to the following equation for plasma with a hemoglobin concentration of 0 g/dL:

\[
BE_{plasma} = (0.0307 \times P_{CO_2} \times 10^{0.037 \times \left(7.40 - pH\right)}) - 24.4 + (7.7 \times \left(pH - 7.40\right))
\]

Identifying unmeasured strong anions in plasma is clinically valuable, because it alerts clinicians to lactic acidosis, ketoacidosis, or uremic anions as well as potential ingestion and absorption of toxic chemicals that are fully dissociated at physiologic pH. Analysis of the results of the study reported here suggests that the SIG provides the best method for quantifying unmeasured strong anion concentration in bovine plasma. Although the Fencl base-excess approach has been applied to acid-base disturbances in domestic animals, the study reported here appears to be only the third study to formally evaluate the Fencl method for quantifying unmeasured strong anions in plasma. In a study in pigs, the Fencl method was found to be a biased and imprecise predictor of unmeasured strong ion concentration, whereas in a study of critically ill pediatric patients, plasma lactate concentration was better correlated with BEba by use of the Fencl base-excess approach (R², 0.55) than with anion gap (R², 0.41), which was similar to the results of the study reported here in critically ill cattle. Because the Fencl base-excess approach is based on laboratory data similar to that used for the SIG but has reduced explanatory power (lower R²; Fig 2, Table 1) while ignoring the effect that pH has on net protein and phosphate charge and assuming a constant potassium concentration, SIG appears to provide the best method for quantifying unmeasured strong anion concentration in bovine plasma.

Value of the slope for the SIGalbunin equation was not significantly different from –1, but the intercept value was significantly different from 0, indicating that the sum of the positive charges on unmeasured strong cations (calcium and magnesium) exceeded the sum of the negative charges on unmeasured strong anions (sulfate, ketoacids, volatile fatty acids, and nonesterified fatty acids) by approximately 3 mEq/L (Table 1). We reported a similar finding in horses. Accordingly, the following equation is recommended for quantifying the unmeasured strong anion concentration in bovine plasma:

\[
SIG = (7.6 \times \text{[albumin]/(1 + 10^{7.06 – pH}) – 3}) – \text{anion gap}
\]

The A tot and K a values calculated in the study reported here suggest that the negative charge of protein and phosphate in bovine plasma at pH 7.43 is 17.5 mEq/L, assuming total protein concentration is 7.0 g/dL. The plasma bicarbonate concentration is 26.4 mM/L when pH is 7.43 and P CO2 is 43 mm Hg. The electroneutrality equation from the simplified strong ion model (SIG – [HCO3] – [A] = 0) indicates that SIG is approximately equal to [HCO3] + [A] = 43.9 mEq/L. Therefore, the calculated A tot and K a values are consistent with the initial assumption that SIG is approximately 44 mEq/L. The calculated A tot and K a values also suggest that net protein charge in bovine plasma is 14.6 mEq/L, assuming that the typical net phosphate charge is 2.9 mEq/L (equivalent to a phosphate concentration of 5 mg/dL at pH 7.43; Appendix). This estimate for the net protein charge of bovine plasma was slightly greater than that obtained by Darrow and Hartman (12.1 mEq/L) by use of the assumptions that albumin concentration is 3.3 g/dL, total protein concentration is 7.0 g/dL, and pH 7.43. Details were not provided concerning the experiments performed by those authors, but they did indicate that they suspected their values were probably not correct. Accordingly, analysis of results of the study reported here suggests that the normal net charge of bovine plasma protein is approximately 15 mEq/L.

A value for A tot was estimated from plasma albumin or total protein concentration, using the assumption of a linear relationship with a value of 0 for the intercept between A tot and the albumin or total protein concentration. This approach, although validated, is an oversimplification of the relationship between A tot and its 3 main constituents (concentrations of albumin, globulin, and inorganic phosphate), because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate, because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate, because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate, because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate, because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate, because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate, because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate, because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate, because it assumes equal contributions to A tot from albumin, globulin, and inorganic phosphate.
the value of $A_{tot}$ and provides a more accurate prediction of the strong ion gap than does total protein concentration, it appears reasonable to use plasma albumin concentration as an index for the true value for $A_{tot}$ (Table 1). It remains to be determined whether compartmentalizing the value for $A_{tot}$ into albumin, globulin, and phosphate components will improve the clinical utility and accuracy of the strong ion approach to acid-base balance.

The correct units for $A_{tot}$ are millimoles per liter, not milliequivalents per liter.16 Millimoles refers to dissociable groups capable of donating or accepting a proton, because the strong ion approach assumes that plasma nonvolatile buffer mass, rather than charge, is conserved.16 Moreover, using the units of milliequivalents per liter for $A_{tot}$ is incorrect, because the value for $A_{tot}$ is less than that in units of millimoles per liter, because the assumption of mass conservation indicates the following: $[A_{tot}] = [HA] + [A^-]$, where all values are reported in millimoles per liter. Because another assumption in the strong ion approach is that $A^-$ is a univalent base and $HA$ is not ionized,16 the following equation also is true:

$$[A_{tot}] = (0 \times [HA]) + (-1 \times [A^-]) = [A^-]$$

where the value of $A^-$ is in millimoles per liter. It therefore follows that the value for $A_{tot}$ in millimoles per liter is greater than the value for $A_{tot}$ in milliequivalents per liter.

The mechanism for acid-base disturbance in calves with diarrhea continues to be actively investigated. Although acidemia and metabolic acidosis in diarrheic calves were originally attributed to severe dehydration and hyperlactatemia, it has been documented that severe dehydration (> 12% of body weight) produces only mild acidemia and small increases in plasma lactate concentration12 and that acidemia results primarily from decreased SID in response to hyponatremia and increased strong anion concentration such as D-lactate from ruminal or intestinal fermentation of milk.11,13 Appropriate application of the strong ion approach and calculation of the SIG in calves with diarrhea would improve our understanding of the mechanism for metabolic acidosis, thereby facilitating treatment. The strong ion approach should also prove useful in clarifying the mechanism for the effects of nutrition on acid-base balance; the effects of dietary cation-anion balance on rumen pH, serum electrolyte concentrations, and acid-base balance in prevention of hypocalcemia in dairy cows; and the acid-base effects of increased plasma β-hydroxybutyrate and acetate concentrations in cows with primary acidemia.7

Finally, the Stewart strong ion model states that plasma pH is a function of 8 factors (SID+, PCO2, $A_{tot}$, $K_1^+$, $S_K$, $K_w$, $S_K$, and $K_w$), whereas the simplified strong ion model states that plasma pH is a function of 6 factors (SID+, PCO2, $A_{tot}$, $K_1^+$, $S_K$, and $K_w$). The spider plot includes 2 additional factors ($K_3$ and $K_w$) in the Stewart strong ion model1 (Fig 3). Although $K_3$ and $K_w$ have been documented algebraically to be redundant when the strong ion approach is used,9 evaluation of the spider plot provides strong graphic evidence that the value for $K_3$ or $K_w$ does not alter pH under physiologic conditions, indicating that neither factor influences plasma pH, and, therefore, should be neglected. When comparing models, the preferred model should have greater explanatory power, mathematical simplicity, or theoretic elegance.10 Because the simplified strong ion model is mathematically simpler than the Stewart strong ion model and has similar explanatory power,14 application of Occam’s razor indicates that the simplified strong ion model should be preferred when the strong ion approach is used.

### References

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