Determination of Strong Ion Difference and Anion Gap from blood biochemical parameters in Lactating Cow

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List of Abbreviations

SID	: Strong Ion Differences ($SID_{3/4/5}$, the subscript means the number of ion used to calculate strong ion differences)
A_{tot}	: Total plasma concentration of nonvolatile weak acids
K_a	: Effective dissociation constant for plasma weak acids
FVM	: Faculty of Veterinary Medicine
CVASU	: Chattogram Veterinary and Animal Sciences University

Abstract

The objectives of this study are to determine serum strong ion differences (SID) and anion gap (AG) of 8 high yielding lactating dairy cows with a history of inappetence and drop of milk production. Blood biochemical data of 8 cows were collected from diagnostic reports done at the Department of Physiology, Biochemistry and Pharmacology, Chattogram Veterinary and Animal Sciences University. Concentrations of quantitatively important strong ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-) and nonvolatile buffer ions (total protein and phosphate) were determined and a fixed L-lactate value was used. Mean (±SD) of Strong ion difference (SID) was determined by calculating the differences between measured strong anion and cation concentrations (SID_3, 37.56 mEq/L; SID_4, 37.02 mEq/L; SID_6, 40.14 mEq/L). Mean value of A_{tot}, total plasma concentration of nonvolatile weak acids was calculated (A_{tot}, 28.10 mmol/L) and this value along with a fixed value of K_a, effective dissociation constant for plasma weak acids and pH is then used to calculate concentrations of HCO_3^-. Anion Gap is then determined and the mean value of anion gap (AG) is 16.62 mEq/L, which is within the normal reference range. Although acid-base abnormalities are frequently present in sick animals. Measuring SID and AG will not only help in explaining the underlaying disease mechanisms of Acid-base disorders but also will help in proper treatment protocols.

Keywords: Strong ions, strong ion differences, anion gap

Chapter 1: Introduction

The difference between positively and negatively charged strong ions in plasma is known as the Strong Ion Difference (SID). At physiologic pH, strong ions are cations and anions that exist as charged particles separated from their companion ions. As a result, these ions are considered "strong" since their ionization state is not affected by pH. Rather than categorizing acid-base diseases into metabolic vs. respiratory acidosis/alkalosis like the Henderson-Hasselbalch equation does, this method was devised to help pinpoint the mechanism of the disorder (Lloyd, 2004). Strong cations predominate in the plasma at physiologic pH leading to a net positive plasma charge. SID can be estimated as follows: SID = [strong cations] – [strong anions] = $[Na^+ + K^+ + Ca^{2+} + Mg^{2+}] – [Cl^- + lactate^-]$

SID-increasing disturbances raise blood pH (alkalosis), whereas SID-decreasing disturbances lower plasma pH (acidosis). The number of positive charges equals the sum of negative charges, according to the law of electroneutrality. As a result, the SID must equal the sum of the body's weak anions (such as bicarbonate, albumin, and phosphate). There are unmeasured anions since the quantity of positive and negative ions in a solution must be equal (SID = 0). Increased SID (>0) implies alkalosis (increase in unmeasured anions), decreased SID (<0) suggests acidosis, and plasma is generally slightly alkaline (given that SID is about 40mEq/L).

Increased SID may be caused by:

- Dehydration (contraction alkalosis) due to increased Na^+
- Chloride loss (e.g., aggressive nasogastric suctioning with loss of HCl)

Decreased SID may be caused by:

- Free water excess (dilutional acidosis) due to decreased Na^+
- Severe diarrhea due to loss of K^+ and Na^+
- An increase in unmeasured anions such as lactate (e.g. lactic acidosis) or ketoacids (e.g. diabetic ketoacidosis)

pH and bicarbonate concentration ([HCO_3]) of an aqueous biological solution are determined by three independent variables: 1) carbon dioxide tension (P_{Co2}); 2) strong ion difference (SID), which is the difference between the charge of strong cations (sodium, potassium, calcium, magnesium) and strong anions (chloride, lactate, sulfate, ketoacids, non-esterified fatty acids, and many others) that are completely dissociated in biologic solutions, and 3) the total weak acid concentration (A_{tot}) which includes all non-volatile weak acids in the system, such as proteins and inorganic phosphates that are modelled as having a single effective dissociation constant (K_a) (Staempfli & Constable, 2003).

Plasma proteins provide the major contribution to A_{tot} and therefore plasma protein concentration independently affects acid-base balance. The role of plasma protein concentration in acid-base balance is well recognized in human and veterinary medicine, with hypoproteinemia and hyperproteinemia causing alkalemia and acidemia, respectively (Staempfli & Constable, 2003)

A_{tot} refers to the total plasma concentration of inorganic phosphate, serum proteins and albumin (weak non-volatile acids), it can be defined as follows: $A_{tot} = [Pi_{tot}] + [Pr_{tot}] +$ [albumin]. A_{tot} abnormalities (non-volatile weak acids) happens due to excess or deficit of inorganic phosphate and excess or deficit of albumin.

The anion gap reflects the difference in the serum (plasma) concentrations of the "measured" cations and "measured" anions and is calculated using the following formula: Anion gap = $(Na^+ + K^+) – (Cl^- + HCO_3^-)$. Changes in anion gap are used primarily to distinguish between causes of a metabolic acidosis. Various pathophysiologic contributes to the increase and decrease of Anion Gap. These are as follows:

Increased Anion Gap

- Titration or high anion gap metabolic acidosis*:* This is due to accumulation of a non-carbonic (nonvolatile) acid, such as L- or D-lactate, ketones and uremic acids (e.g., sulfates, phosphates).
- Alkalemia*:* Loss of protons (H^+) from plasma proteins (particularly albumin) in an attempt to buffer the increase in bicarbonate, increases their net negative charge.

- Increased albumin*:* This may increase the anion gap because it is an “unmeasured” anion, e.g., dehydration, increased albumin production.
- Decreased “unmeasured” cations

Decreased Anion Gap

- Decreased albumin
- Administration of bicarbonate-rich fluids.
- Increased “unmeasured” cations
- Acidemia: Protons released from accumulated acids are buffered by plasma proteins (e.g., albumin), which decreases their normal negative charge.

  The high yielding dairy cows suffer from electrolytes imbalance due large amount of milk let down and change the internal milieu of the body. However, this type of study is very limited. Considering the above background, the present study aim is to estimate the strong ion differences and the correlation with certain blood biochemical parameters also to estimate Anion Gap using a calculated value of HCO_3^- in serum of high yielding lactating cow

Chapter 2: Materials and Methods

2.1. Blood and Plasma Analyses

The data used in this study were analyzed and were recorded at the Department of Physiology, Biochemistry & Pharmacology, FVM, CVASU, dated between April 2019 and November 2019. The blood biochemistry of these data provides the value of estimation of $[Na^+]$, $[K^+]$, $[Ca^{2+}]$, $[Mg^{2+}]$, $[Cl^-]$, Phosphorous, Glucose, Total Protein in blood, these data were obtained and used in this study.

2.2. Calculation of SID

SID was estimated using 3 methods: $SID_3 = ([Na^+] + [K^+]) - ([Cl^-])$; $SID_4 = ([Na^+] + [K^+]) - ([Cl^-] + [L\text{-lactate}])$; $SID_6 = ([Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}]) - ([Cl^-] + [L\text{-lactate}])$. Where $[Na^+]$, $[K^+]$, $[Ca^{2+}]$, $[Mg^{2+}]$, $[Cl^-]$, Phosphorous, Glucose, Total Protein were analyzed and fixed value of L-lactate was used 0.54 mM/L according to Figueiredo et al., (2006), where they finds the median plasma L-lactate was 0.54 mM/L (interquartile range, 0.42-0.74) in healthy lactating cows.

2.3. Estimation of A_{tot}

A_{tot} is the total plasma concentration of nonvolatile weak acids. The formula to estimate A_{tot} from plasma albumin & phosphate is as follows: $A_{tot} = 0.325275*[albumin] + 2*[phosphate]$. Here, albumin as estimated from total protein, as albumin constitutes 60% total protein & phosphate is calculated from as phosphorous, since the phosphate (PO_4) molecule is three times as heavy as the P atom, results reported as PO_4 are three times the concentration of those reported as P. Albumin (g/L) = Total protein(g/L) * 0.6 & Phosphate (mmol/L) = Phosphorous * 3

2.4. Calculation of Anion Gap

The anion gap reflects the difference in the serum concentrations of the "measured" cations and "measured" anions and is calculated using the following formula: Anion gap = $(Na^+ + K^+) - (Cl^- + HCO_3^-)$. Na^+, K^+, Cl^- were analyzed and we calculated HCO_3^- from the

following formula: $[HCO_3^-] = SID - (A_{tot} \cdot K_a)/(K_a + 10^{-pH})$, (Constable et al., 2005), K_a, effective dissociation constant for plasma nonvolatile weak acids, for the purpose of estimating HCO_3^- value we take a fixed value of $K_a = 0.9 * 10^{-7}$ & pH = 7.38 (Kellum, 2000).

2.5. Statistical Evaluation

The extracted data as required from the department paper-based recording system were entered into Microsoft Excel 2019 spread sheet. Data were then cleaned for errors and inconsistencies, sorted, coded and checked for integrity in MS Excel 2019. Afterwards, data were exported to STATA/IC 15.1 (StataCorp, 4905, Lakeway Drive, College station, Texas, USA) for conducting the statistical analysis.

Chapter 3: Results

3.1. Estimation of Strong Ion Difference

The means of estimated value have small differences among SID_3 (37.56, mEq/L), SID_4 (37.02, mEq/L), SID_6 (40.14, mEq/L) were obtained along with standard deviation and 95% confidence interval. (Table 1)

Table 1: Summary of mean (±SD) estimated values of SID (n=8) with 95% CI

Measured Method	Mean (±SD)	95% Confidence Interval	
SID_3	37.56 (±12.94)	26.74	48.38
SID_4	37.02 (±12.94)	26.20	47.84
SID_6	40.14 (±12.72)	29.50	50.77

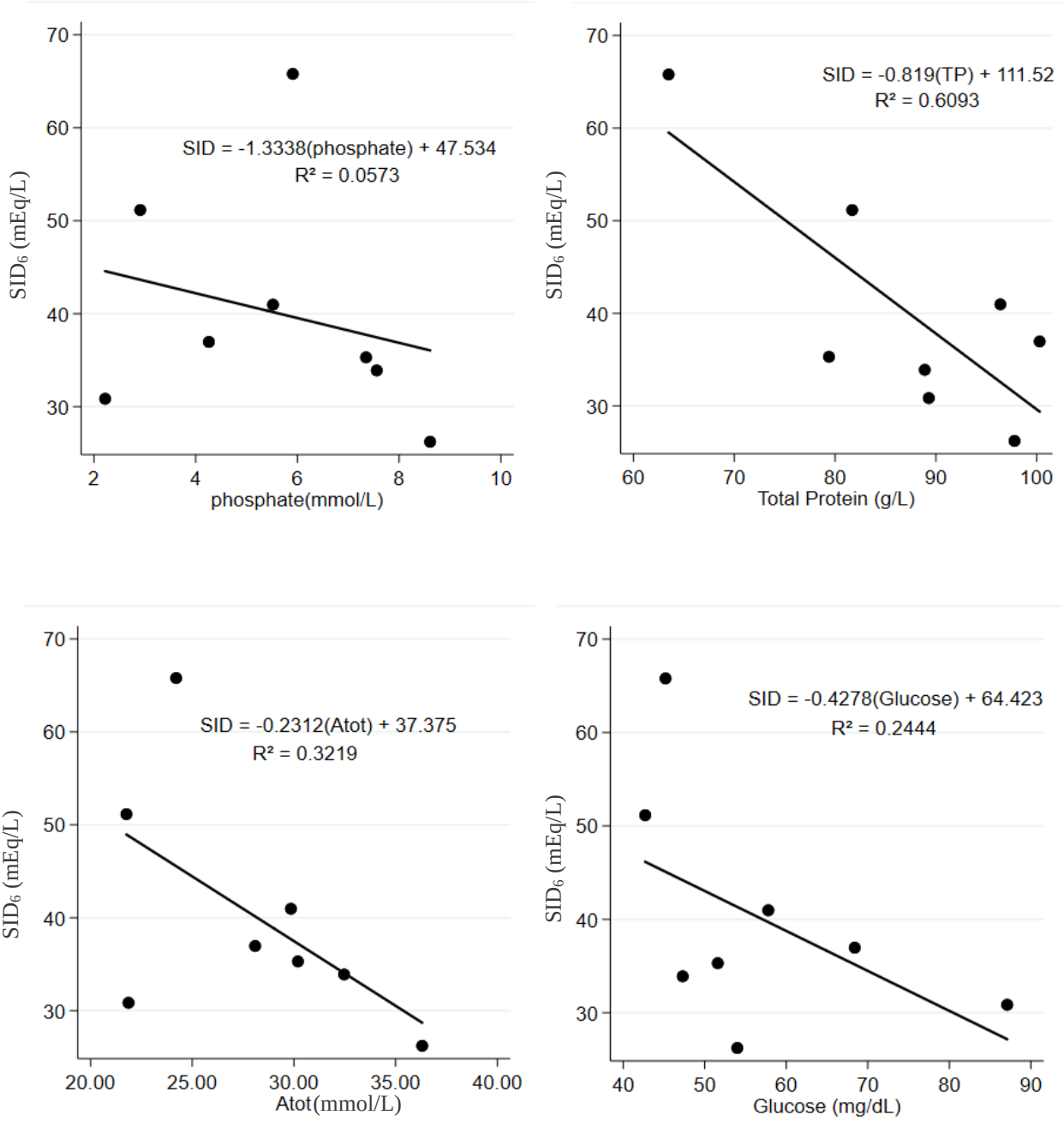
Table 2 shows that estimated SID_6 value was highly negatively correlated with total protein concentration (r = - 0.78; Table 2)

Table 2: Pearson Correlation Coefficients(r) of Selected Variables (using SID_6) (n=8)

	SID_6	TP	Phosphate	Glucose	A_{tot}
SID_6	1.0000	-0.78	-0.24	-0.49	-0.57
TP		1.00	0.02	0.46	0.47
Phosphate			1.00	-0.51	0.89
Glucose				1.00	-0.24
A_{tot}					1.00

The estimated SID_6 has highly inverse correlation with total protein (r = -0.78). There is moderate inverse correlation between SID_6 and A_{tot} (r = -0.57); low inverse correlation between SID_6 and glucose (r = -0.49) & very little inverse correlation between SID_6 and phosphate (r = -0.24) (Figure 1).

Figure 1: Relationship Between Strong ion difference (SID) and Total Protein (TP), Glucose, phosphate concentrations & A_{tot} (n = 8)



3.2. Estimation of A_{tot} & AG

Mean value of A_{tot}, total plasma concentration of nonvolatile weak acids is 28.10 mmol/L and mean value of anion gap (AG) is 16.62 mEq/L (Table 3).

Table 3: Mean (±SD) values of A_{tot} and Anion Gap (AG)

Measured Variable	Mean (±SD)	95% Confidence Interval	
A_{tot}	28.10 (±5.18)	23.76	32.43
AG	16.62 (±3.14)	14.00	19.25

Table 4 shows that estimated AG value was very highly correlated with A_{tot} (r = 0.99) and highly correlated with phosphate concentration (r= 0.87)

Table 4: Pearson Correlation Coefficients(r) of Selected Variables (Using AG) (n=8)

	AG	TP	Phosphate	Glucose	A_{tot}
AG	1.0000	0.49	0.87	-0.17	0.99
TP		1.00	0.02	0.46	0.47
Phosphate			1.00	-0.51	0.89
Glucose				1.00	-0.24
A_{tot}					1.00

The AG is very highly correlated with A_{tot} (r = 0.99). There is high correlation between AG and phosphate (r = 0.87); low correlation between AG and TP (r = 0.49). A_{tot} is also has highly correlated with phosphate (r = 0.89). (Figure 2)

Figure 2: Relationship Between Anion Gap (AG) and Total Protein (TP), Glucose, phosphate concentrations & A_{tot} (n = 8)

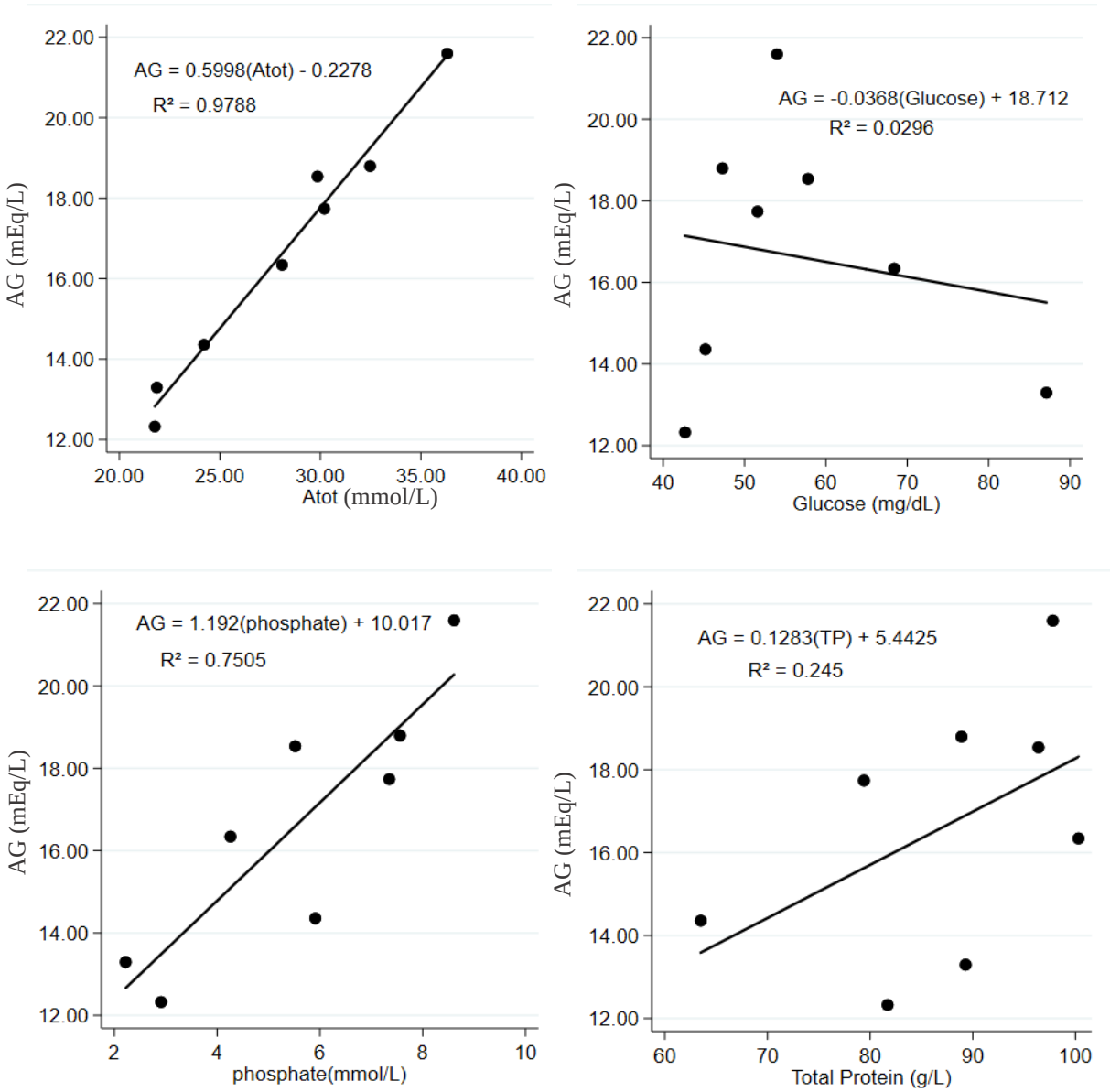


Table 5: Mean (±SD) values of Measured Electrolytes in Cow (n =8)

Measured ion	Mean (±SD)	95% Confidence Interval		Range of Values	Normal Range
Ca	2.08 (±0.58)	1.59	2.56	0.95 – 2.65	2.0–2.8
Mg	1.04 (±.25)	0.83	1.26	0.53 – 1.36	0.6–1.2
P	1.85 (±0.76)	1.21	2.48	0.74 – 2.87	1.8–2.6
Na	143.28 (±14.26)	131.35	155.20	118.5 – 158.7	136–144
K	3.9 (±1.23)	2.87	4.93	2.2 – 6.2	3.6–4.9
Cl	109.61 (±10.91)	100.49	118.73	97.8 - 127	99–107
HCO_3	20.94 (±15.01)	8.39	33.49	1.41 – 49.24	20–30

Table 6: Ca:P & Na:Cl Ratio

Calcium	Phosphorous	Ca:P	Sodium	Chloride	Na:Cl
1.9	0.97	2.53:1	156.5	111.5	1.40:1
1.7	1.97	1.11:1	158.7	99.4	1.60:1
2.45	2.45	1.29:1	148.5	119.8	1.24:1
1.875	1.84	1.32:1	138.6	102.2	1.36:1
2.65	2.87	1.19:1	118.5	101.7	1.17:1
2.5	1.42	2.27:1	127.2	97.8	1.30:1
2.575	2.52	1.32:1	145.8	117.5	1.24:1

Chapter 4: Discussion

The Henderson-Hasselbalch equation has long been used to help veterinarians treat animals with acid-base imbalances. There are three reasons why the Henderson-Hasselbalch equation has been effective in guiding the management of acid-base imbalances in ill animals with or without diarrhea in clinical practice. First, animals with acidemia and low plasma [HCO_3^-] have been treated with an isosmotic sodium bicarbonate solution administered intravenously, with correction of the acid-base imbalance attributed to an increase in plasma [HCO_3^-] rather than an increase in plasma SID after therapy. Second, assuming that bicarbonate distributes in the extracellular fluid space, calculating the standardized base excess or real bicarbonate concentration has provided a quick and reliable approach for determining bicarbonate requirements. Finally, a change in plasma [HCO_3^-] from normal is comparable to a change in plasma SID from normal, as long as plasma, and therefore plasma albumin, globulin, and phosphate concentrations and pH, remain unchanged.

To determine acid-base status using strong ion differences, species-specific values of particular strong ion concentrations are required. The value for strong ion differences was estimated using the strong cations and anions that were measured. Our calculated mean values for SID_3, SID_4, SID_6 in cows were 37.56 mEq/L, 37.02 mEq/L, 40.14 mEq/L (Table 1) which were slightly less than the calculated mean values found by Constable et al., (2005) which were 43.0 mEq/L, 41.1 mEq/L, 45.4 mEq/L respectively.

The estimated mean value for A_{tot} in cows was 28.10 mmol/L with standard deviation of ±5.18 (Table 3) which was slightly higher than the estimated value found by Constable et al., (2005) which was 23.1 mmol/L with standard deviation of ±6.1. In this study, the A_{tot} value was calculated using a formula based on albumin and phosphate ion concentration (Lloyd, 2004).

Calculated Pearson correlation coefficient (Table 2) shows that, SID (using SID_6) has high inverse correlation with total protein (r = -0.78). There is moderate inverse correlation between SID and A_{tot} (r = -0.57), low inverse correlation between SID and glucose (r = -0.49) & very little inverse correlation between SID and phosphate (r = -0.24) (Figure 1).

Estimated mean value of anion gap (AG) in Cows is 16.62 mEq/L which is within the normal range in cows (Constable et al., 1997). Estimated AG value was very highly correlated with A_{tot} (r = 0.99) and highly correlated with phosphate concentration (r= 0.87) (Table 4) (Figure 2). Where Constable et al., (1997) also found a higher correlation between phosphate concentration and AG (r = 0.71). The correlation coefficient value between AG and total protein concentration found by Constable et al., (1997) is r= 0.54, which is slightly higher than our calculated value (r= 0.49).

Changes in the AG of ill animals could be caused by changes in plasma protein, phosphate, or unmeasured strong cation and anion concentrations. In other words, the AG is a nonspecific approach for assessing the unmeasured strong anion concentration in cattle plasma (such as D-lactate or L-lactate).

Limitations

The sample size of this study was small and the animals were not categorized according to their age and diseases. Some variables were assumed and assigned a fixed normal value which may give a less accurate measurement of actual concentration of electrolytes. Previous disease history and case history of the animal, if included, would have helped in interpreting the changes in results of this study.

Conclusion

The strong ion difference is a superior method of monitoring acid-base state by including A_{tot} and free water in detecting metabolic alkalosis and acidosis. Changes in free water change Na^+ and Cl^- proportionately, thus the Cl^- will be normal. However, a gain of free water will induce an acidosis (dilutional) by reducing a strong cation (Na^+), whereas a loss of free water would cause an alkalosis by increasing a strong cation (Na^+). Measurement of SID and AG will help us identifying the underlying mechanism of Acidosis and Alkalosis along with their correlation with plasma biochemical constituents.

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Biography

Rakibul Islam was born on May 7, 1998, to Parul Akter and Abu Sufian. He earned a 5.00 GPA in the Secondary School Certificate Examination in 2013 from Ataturk Model High School in Feni and a 5.00 GPA in the Higher Secondary Certificate Examination in 2015 from Feni Govt. College in Feni. He is now enrolled in a year-long internship program at Chattogram Veterinary and Animal Sciences University in Chattogram, Bangladesh, as part of his Doctor of Veterinary Medicine (DVM) degree curriculum.