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Research article

Determination of strong ion difference and anion gap in serum biochemical markers of the lactating cow

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A B S T R A C T

Acid-base abnormalities are frequently present in sick animals. Measurement of strong ion difference (SID) and anion gap (AG) is not only useful in explaining the underlying disease mechanisms of acid-base disorders but also assist in proper treatment protocols. The objectives of this study were to determine SID and AG in serum of high yielding lactating dairy cows with a history of inappetence and drop of milk production. Data on serum biochemical markers of 8 cows were collected from diagnostic reports preserved at the department of Physiology, Biochemistry and Pharmacology, Chattogram Veterinary and Animal Sciences University. The SID was calculated as SID = [strong cations] – [strong anions] = ($[Na^+]$+ $[K^+]$+ $[Ca^{2+}]$+ $[Mg^{2+}]$) – ($[Cl^-]$+ $[Lactate^-]$) and the AG was calculated as AG =($[Na^+]$+$[K^+]$)-($[Cl^-]$+$[HCO_3^-]$ with all values in mEq/L. Mean value of total plasma concentration of nonvolatile weak acids (A_{tot}) was calculated following the equation, A_{tot} = 0.325275*[albumin] + 2*[phosphate], and the mean value of HCO_3^- was calculated as $[HCO_3^-]$ =SID-(A_{tot}×K_a)/(K_a+10^{-pH}), where K_a = 0.9 * $10^{-7}$and pH = 7.38 . The fixed value of lactate was 0.54 mM/L. The calculated mean values of SID were SID_3, 37.56 mEq/L; SID_4, 37.02 mEq/L; SID_6, 40.14 mEq/L, respectively. Total plasma concentration of non-volatile weak acid was 28.10 mmol/L. And the mean value of anion gap was 16.62 mEq/L. Anion gap was highly correlated with the total plasma ion concentrations of non-volatile weak acids (r=0.99) followed by phosphate (r=0.89) and total protein (r=0.47). In summary, we may conclude that high total plasma concentration of non-volatile weak acids, hyperphosphataemia and high total protein concentrations were commonly associated with lactating cows.

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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, both cations and anions 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. The SID

can be estimated as: SID = [strong cations] – [strong anions] = ($[Na^+ + K^+ + Ca^{2+} + Mg^{2+}]$) – ($[Cl^-]$+ [Lactate$^-$]).

The SID-increasing disturbances raise blood pH (alkalosis), whereas SID-decreasing disturbances lower plasma pH (acidosis). The sum 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 since 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^+ and chloride loss (e.g., aggressive nasogastric suctioning with loss of HCl) whereas decreased SID may be caused by free water access (dilutional acidosis) due to decreased Na^+, severe diarrhea due to loss of K^+ and Na^+ and an increase in unmeasured anions such as lactate (e.g. lactic acidosis) or ketoacids (e.g. diabetic ketoacidosis).

The pH and bicarbonate concentration ($[HCO_3^-]$) of an aqueous biological solution are determined by three independent variables: 1) carbon dioxide tension (P_{Co2}); 2) SID, i.e., 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) which remains completely dissociated form 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 and 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) which can be defined as follows: A_{tot} = [Pi_{tot}] + [Pr_{tot}] + [albumin]. A_{tot} abnormalities (non-volatile weak acids) happen 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 pathophysiology contributes to the increase and decrease of anion gap, like, increased anion gap due to accumulation of a non-carbonic (nonvolatile) acid, such as L- or D-lactate, ketones and uremic acids (e.g., sulfates, phosphates); alkalemia, i.e., loss of protons (H^+) from plasma proteins (particularly albumin) in an attempt to buffer the increase in bicarbonate, increase in their net negative charge, increased albumin because of an "unmeasured" anion, e.g., dehydration and increased albumin production due to decreased "unmeasured" cations. On the contrary decreased AG caused decreased albumin, administration of bicarbonate-rich fluids, i.e., increased "unmeasured" cations acidemia because of protons released from accumulated acids buffered by plasma proteins (e.g., albumin), which decreases their normal negative charge. The high yielding dairy cows suffer from electrolytes imbalance due to large amount of milk let down and change in internal milieu of the body. In Bangladesh, the number of high yielding commercial dairy cows increasing and the farmers face problems with these types of lactating cows especially at post-partum period in early lactation. The common complains of the farmers are inappetence and drop of milk production of their recently calved cows (Tanzin et al. 2019). Although, these symptoms are common there is no study in the context of Bangladesh. Considering the above backgrounds, aims of the present study were to estimate the SID and the correlation with certain blood biochemical parameters and also to

estimate the AG using a calculated value of HCO_3^- in serum of high yielding lactating cow.

2. MATERIALS AND METHODS

Blood and plasma analyses

The data used in this study were analyzed and were recorded at the Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University (CVASU), dated between April 2019 and November 2019. The blood biochemistry of these data provided the value of estimation of $[Na^+]$, $[K^+]$, $[Ca^{2+}]$, $[Mg^{2+}]$, $[Cl^-]$, phosphorous, glucose and total protein in blood serum.

Calculation of SID

The 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. A fixed value of L-lactate was used as 0.54 mM/L according to Figueiredo et al. (2006), who reported the median plasma L-lactate was 0.54 mM/L (interquartile range, 0.42-0.74) in healthy lactating cows.

Estimation of total plasma concentration of non-volatile weak acids (A_{tot})

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

Calculation AG

The *anion gap* reflects the difference in the serum concentrations of the "measured" cations and "measured" anions and was 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: $[HCO_3^-]=SID-(A_{tot}\times K_a)/(K_a+10^{-pH})$,(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 $K_a = 0.9 * 10^{-7}$and pH = 7.38 (Kellum, 2000).

Statistical analysis

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 descriptive statistical analysis such as Mean±SD, 95% Confidence Interval and Pearson Correlation Coefficient were performed. P-value ≤0.05 was considered as significant difference.

3. RESULTS

Measured electrolytes in cow

The mean (±SD) of serum calcium (2.08 ±0.58, mmol/l), magnesium (1.04 ±.25,mg/dl), potassium (3.9±1.23, mmol/l), were found lower than the normal limit. The mean values of other parameters including phosphorus, sodium, chloride and bicarbonate were found within normal limit.

Estimation of SID

The means of estimated value have small differences among SID3 (37.56, mEq/L), SID4 (37.02, mEq/L), SID6 (40.14, mEq/L) were obtained along with standard deviation and 95% confidence interval (Table2). The estimated SID6 had highly inverse correlation with total protein (r = -0.78). There was moderate inverse correlation between SID6 and A_{tot} (r = -0.57). Low inverse correlation between SID6 and glucose (r = -0.49) and very little inverse correlation between SID6 and phosphate were noticed (r = -0.24) (Table-3). Table 3 shows that estimated SID6 value was highly negatively correlated with total protein concentration (r=-0.78).

Table 1. Measured electrolytes in cow (n =8)

Measured ion	Mean (±SD)	95% CI		Range of values	Normal range (Kaneko et al., 1997)
Calcium(mmol/l)	2.08 (±0.58)	1.59	2.56	0.95 – 2.65	2.43-3.10
Magnesium (mg/dl)	1.04 (±.25)	0.83	1.26	0.53 – 1.36	1.8-2.3
Phosphorus(mmol/l)	1.85 (±0.76)	1.21	2.48	0.74 – 2.87	1.82-2.10
Sodium(mmol/l)	143.28 (±14.26)	131.35	155.20	118.5 – 158.7	132-152
Potassium(mmol/l)	3.9 (±1.23)	2.87	4.93	2.2 – 6.2	3.92-5.8
Chloride (mmol/l)	109.61 (±11)	100.49	118.73	97.8 - 127	97-111
Bicarbonate(mmol/l)	20.94 (±15)	8.39	33.49	1.41 – 49.24	17-29

Table 2. Estimated values of strong ion difference (n=8)

Measured Method	Mean (±SD)	95% CI	
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

SID, strong ion difference.
[SID3]=([Na^+]+[K^+])-[Cl^-].
[SID4]=([Na^+]+[K^+])-([Cl^-]+[L-lactate]).
[SID6]=([Na^+]+[K^+]+[Ca^{2+}]+[Mg^{2+}])-([Cl2]+[L-lactate]).
95% CI, 95% Confidence Interval

Table 3. Pearson correlation coefficients® of selected variables (using SID_6) (n=8)

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

[SID6]=([Na^+]+[K^+]+[Ca^{2+}]+[Mg^{2+}])-([Cl2]+[L-lactate]).
Atot, total plasma concentration of nonvolatile weak acids

Estimation of A_{tot} and AG

Mean value of A_{tot}, total plasma concentration of nonvolatile weak acids was 28.10 mmol/L and mean value of anion gap (AG) was 16.62 mEq/L (Table 4). The AG was very highly correlated with A_{tot} (r = 0.99). There was high correlation between AG and phosphate (r = 0.87); low correlation between AG and TP (r = 0.49). Furthermore, A_{tot} was also highly correlated with phosphate (r = 0.89) (Table 5).

4. DISCUSSION

The Henderson-Hasselbalch equation has long been used to help veterinarians in treatment 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 [HCO3–] have been treated with an isosmotic sodium bicarbonate solution administered intravenously, with correction of the acid-base imbalance attributed to an increase in plasma [HCO3–] 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 [HCO3–] 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.

Table 4. Total plasma concentrations of non-volatile weak acids (A_{tot})and Anion Gap (AG)

Measured Variable	Mean (±SD)	95% CI		Normal range
A_{tot}	28.10 (±5.18)	23.76	32.43	
Anion gap (mEq/l)	16.62 (±3.14)	14.00	19.25	14-20 (Constable et al., 1997)

A_{tot}, total plasma concentration of nonvolatile weak acids
Anion gap=($[Na^+]$+$[K^+]$)-($[Cl^-]$+$[HCO3^-]$.
95% CI, 95% Confidence Interval

Table 5. Pearson Correlation Coefficients(r) of Selected Variables (Using AG) (n=8)

	Anion gap	Total protein	Phosphate	Glucose	A_{tot}
Anion pap	1.0000	0.49	0.87	-0.17	0.99
Total protein		1.00	0.02	0.46	0.47
Phosphate			1.00	-0.51	0.89
Glucose				1.00	-0.24
A_{tot}					1.00

Anion gap=($[Na^+]$+$[K^+]$)-($[Cl^-]$+$[HCO3^-]$.
A_{tot}, total plasma concentration of nonvolatile weak acids

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_6in cows were 37.56 mEq/L, 37.02 mEq/L, and 40.14 mEq/L, respectively which were slightly lower 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) showed that, SID (using SID_6) had high inverse correlation with total protein (r = -0.78). There was moderate inverse correlation between SID and A_{tot} (r = -0.57), low inverse correlation between SID and glucose (r = -0.49) and very little inverse correlation between SID and phosphate (r = -0.24).

Estimated mean value of 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), which is coincided with earlier findings who reported highly correlation between phosphate concentration and AG (r=0.71) Constable et al. (1997). The correlation coefficient value between AG and total protein concentration was (r= 0.54) reported found by Constable et al. (1997), which is slightly higher than the present 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. The AG is increased with situations of metabolic acidosis and it represents anions such as Cl−, proteins, phosphates, sulfates and organic anions (Hernández et al., 2020). Its measurement is also considered as the nonspecific approach for assessing the unmeasured strong anion concentration in cattle plasma (such as D-lactate or L-lactate). The range of AG for adult animals varies for different species, with published values of 8 to 13 mEq/L (horse) (Cosset, 1983), 14 to 20 mEq/L (cow) (Shull, 1978), 17 to 29 mEq/L (sheep) (Fubini et al. 1991) and 15 to 25 mEq/L (dog) (Shull, 1978). Previous study shows that an AG greater than 30 mEq/L in critically ill cattle with the increase in AG being attributed to an increase in blood lactate and ketoacid concentration, as well as anions associated with

uremia (Constable et al. 1997). Besides strengths of the study, there are some limitations including small number of sample size, 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.

The SID is a superior method of monitoring acid-base state by assessing total plasma concentration of non-volatile weak acids and free water in metabolic alkalosis and acidosis. The changes in free water alter the proportion of Na+ and Cl^-, 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.

5. CONCLUSION

In summary, we may conclude that high total plasma concentration of non-volatile weak acids, hyper-phosphataemia, and total protein strongly influence the anion gap in lactating animals.

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