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Accuracy of direct vs calculated ionized calcium in treatment planning for transition cow medical care: A review of 104 dairy cows

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Abstract

A retrospective study was performed to assess the relationship between serum ionized calcium and calculated serum ionized calcium in dairy animals. Results of 104 apparently healthy dairy animals presented to the Large Animal Medicine Referral Clinic of the Veterinary College and Research Institute, Orathanadu were examined by regression analysis. Analysis of Serum ionized Calcium (iCa), Total Calcium (tCa), Total protein (TP), and albumin concentrations were measured in all samples. Serum concentrations of iCa were measured anaerobically by use of an ion-selective electrode and serum concentrations of tCa, TP, and albumin were measured by use of an automated biochemistry analyzer. Correlation between Total protein (TP), and albumin to the calculated ionized calcium was performed using Pearson correlation and results were interpreted. The predicted low serum ionized calcium values were not due to changes in pH but were associated with hypoalbuminaemia. Adjusted total calcium or total calcium concentrations should not be used for predicting ionized calcium status in dairy animals. The present study revealed that direct measurement of ionized calcium concentration is necessary for accurate assessment of calcium status and also it was concluded that immediate determination of serum iCa in dairy cattle is the ideal to monitor calcium status in transition cows.

Keywords: Ionized calcium, hypocalcemia, dairy animals, metabolic diseases

Introduction

Calcium monitoring is essential in Transition cow health care. Serum calcium exists in 3 fractions: ionized (iCa), complexed (cCa), and protein-bound (pCa). Serum total calcium concentration (TCa) has traditionally been used to assess calcium abnormalities in dairy cows due to its ease availability. Even though iCa is biologically active, clinicians rely on serum tCa measurements to predict iCa status (Patricia and Dennis, 2010) [7]. Variations in pCa or cCa can render the tCa concentration abnormal, even though the iCa fraction is within normal limits. Since some serum tCa is protein-bound, it had been suggested to “adjust” tCa relative to serum total protein (TP) or albumin concentration to improve diagnostic interpretation of tCa, especially when TP or albumin alterations are present (Claire *et al.*, 2009) [8]. Before the development of ion-selective electrodes, researchers commonly used equations to adjust the tCa value on the basis of measured concentrations of total protein (TP) or albumin Caprita and Caprita (2004) [3]. Direct measurement of the ionised fraction of calcium in serum is generally accepted as the best way of detecting disturbances of calcium homeostasis in transition cows (George *et al.*, 1986) [11]. To our knowledge, the adjustment equations for serum tCa concentration have never been verified through comparison with actual iCa measurement in dairy animals. Thus, it is unknown whether serum tCa concentration or adjusted tCa concentration accurately predicts iCa concentration. Hence, the present study was undertaken whether the albumin- adjusted calcium and Total protein adjusted calcium values of better diagnostic accuracy than that of direct measurement of serum ionized calcium using selective ion electrodes.

Materials and Methods

Study plan

From October 2016 to September 2018, samples of 104 apparently healthy cross bred dairy animals which were in transition periods were presented to the Large Animal Medicine Referral Clinic of Veterinary College and Research Institute, Orathanadu was assessed and reviewed. The total calcium concentration on 104 serum samples from Cross Bred dairy animals was adjusted using published equations for adjusting calcium levels for abnormal total protein or albumin concentration. Correlations between ionized calcium and total calcium or adjusted total calcium, total calcium, and total protein, and total calcium and albumin were calculated. Diagnostic discordance between total calcium or adjusted total calcium and ionized calcium was determined. The selected animals were with no obvious clinical disturbance of calcium homeostasis or acid base balance. About 10 ml of blood was collected from jugular venipuncture from all those animals. These samples were handled anaerobically from blood sampling to determination of ionized calcium by the electrode method, and were handled aerobically during the determination of other parameters as per the standard protocol by Lena *et al.* (2014) [9].

Analysis of sample

Ionized calcium was measured by an ion-selective electrode method on Electrolyte Analyzer (Clinisys, All Care Electrolyte Analyzer, India). We did not adjust the ionized calcium to a mean serum pH of 7.4 since recent studies have suggested that unadjusted ionized calcium levels are a more valid and physiologically relevant marker of calcium homeostasis. The serum total protein (TP) was determined with the biuret reagent method as described by Cannon *et al.* (1974) [2], using automated analyzer. The serum albumin concentration was determined using modified bromocresol green colorimetric method as described by Doumas *et al.* (1981) [6]. Determination of tCa concentration in serum was carried out using automatic analyzer.

Formula for calculated parameters as per Toffaletti, J. G. (2011) [1]

Ionized calcium and corrected total calcium were calculated (in mg/dL) with the following formula:

1. Albumin adjusted serum ionized Calcium (iCa) = $[0.9 + (0.55 \times \text{tCa} - 0.3 \times \text{albumin})]$
2. Total Protein adjusted serum ionized Calcium (iCa) = $(6 \times \text{tCa} - \text{TP}/3)/(6 + \text{TP})$
3. Albumin adjusted Corrected Calcium (ctCa) = $\text{tCa} - 0.707 \times (\text{albumin} - 3.4)$

Formula for Calculated Free Calcium as per Sava *et al.*, (2005) [4]

1. % Protein bound = $0.8 \times \text{Albumin (g/L)} + 0.2 \times \text{Globulin (g/L)} + 3$
2. Calculated Free Calcium (mg/dl) = Total calcium - Protein bound Calcium
3. Calculated Free Calcium in mmol/Litre = Calculated Free Calcium (mg/dl) $\times 0.25$

Serum components were expressed as means and standard deviation and were subjected to statistical analysis.

Results and Discussion

One hundred and four apparently healthy cross bred transition cows were tested and values for measured and calculated biochemical parameters are illustrated in table 1. Corrected calcium formula in routine clinical use does not accurately reflect ionized calcium in dairy animals. Some reports recommend the use of albumin-corrected total Ca for routine clinical interpretation of Calcium and the use of ionized Ca is recommended when more exact values are required (Nusrat *et al.*, 2015) [10]. Use of the formula developed by Sava *et al.*, showed overestimation for the prevalence of normocalcemia and underestimation for the prevalence of hypocalcemia. Use of an equation to adjust the tCa concentration on the basis of TP or albumin concentration resulted in an overestimation for the prevalence of hypercalcemia and underestimation for the prevalence of hypocalcemia. The use of an adjustment equation increased the amount of misdiagnosis, with an incorrect diagnosis. The data provided by George *et al.* (1986) [11] and the many studies that support them clearly indicated that ionized Ca is the test of choice in nearly all-medical diagnostic and treatment situations. Hence direct measurement of ionized calcium is highly essential method of choice for accurate assessment of calcium status in productive animals.

Accurate determination of serum iCa is dependent on careful sample collection, transport, and timely analysis to minimize pH changes. The International Federation of Clinical Chemistry (IFCC) recommendations on sampling, transport, and storage for the determination of iCa in human serum has determined that anaerobic collection, filling tubes completely with blood, allowing clot formation at room temperature followed by centrifugation, and separation of serum from erythrocytes within 1 hour minimizes the effect of lactic acid formation on pH.

Among two formulas, calculated free calcium as per Sava *et al.*, (2005) [4] had a slightly better correlation with serum iCa concentration than the formula developed by Toffaletti, J. G. (2011) [1] when the formulas subjected to bovines. Among 104 animals 82.69% of transition cows misrepresented or overestimated the ionized calcium by using calculated formulas.

Table 1: Means and standard deviation of the different Variances

Variances	Units	Mean \pm Standard Deviation
Total Protein	g/dL	8.77 \pm 1.10
Albumin	g/dL	3.02 \pm 0.48
Globulin	g/dL	5.75 \pm 1.45
Calcium	mg/dL	10.36 \pm 1.61
Albumin adjusted Serum ionized Calcium	mg/dL	5.69 \pm 0.86
Total Protein adjusted Serum ionized Calcium	mg/dL	4.04 \pm 0.75
Albumin adjusted Corrected Calcium	mg/dL	10.63 \pm 1.56
Calculated Ica*	mmol/L	1.42 \pm 0.21
% Of Protein Bound	%	6.57 \pm 0.22
Calculated Free Calcium	(mg/dl)	3.80 \pm 1.57
Calculated Free Calcium**	mmol/L	0.95 \pm 0.39
Measured Ica	mmol/L	1.02 \pm 0.16

* indicates calculated parameters as per Toffaletti, J. G. (2011) [1]

** indicates Calculated Free Calcium as per Sava *et al.*, (2005) [4]

Table 2: Pearson correlation for measured ionized calcium (iCa)

Parameters	R value	P value	Correlation
Albumin	0.8234	0.00001	Strong positive
Total Protein	-0.4865	0.00001	Weak negative
Albumin adjusted corrected calcium	0.2338	0.016909	Weak positive
Calculated free calcium	0.2864	0.003204	Weak positive

*. Correlation is significant at the 0.05 level (2-tailed).

There was a strong positive correlation between measured iCa calculated with albumin than Total protein and albumin adjusted corrected calcium. The values based on albumin concentration are the most suitable, since 80-90% of protein bound calcium is an albumin chelate. Changes in plasma albumin can alter total calcium independently of free calcium. When the serum albumin level is low, a higher percentage of total serum calcium will be free and metabolically active, so that even when tCa is low, it is not always a metabolically hypocalcemia. Thus, to correct for an abnormally high or low serum albumin, ctCa can be used. Our study illustrated that calculated iCa from concentrations calculated from tCa and albumin are better indicators of calcium homeostasis than total protein adjusted serum ionized calcium and tCa concentrations alone. But there are not best indicators of calcium status of animal due to overestimation or underestimation of calcium leading to misdiagnosis of the case.

In present scenario the correlation between measured total calcium and total protein and albumin was considerably positive but weakly correlated with calculated free calcium, while measured free calcium showed a negligible correlation with total protein and albumin. Findings of the present study were also corroborated by the work of Sava *et al.* (2005) [4]. Hence, direct measurement of ionized calcium concentration is necessary for accurate assessment of calcium status and also it was concluded that immediate determination of serum iCa in dairy cattle is the ideal to monitor calcium status in transition cows.

Conclusion

Based on the results of this study, it was concluded that immediate determination of serum iCa in dairy cows is the ideal than Calculated Ica from concentrations calculated from available formulas for bovines. Measurement of free calcium by ion Selective Electrode is a simple, rapid and accurate method for assessing calcium status with scrupulous anaerobic sampling, free calcium may be a better indicator than presently used total calcium for assessing calcium status in serum.

References

1. Toffaletti JG. Ionized Calcium, in Clinical Chemistry: Theory, Analysis, Correlation, 5th ed. Kaplan, L.A. and Pesce AJ Mosby Elsevier, St. Louis, Mo., 2011.
2. Cannon DC, Olitzky I, Inkpen JA. Proteins. In: Henry RJ, Cannon DC, Winkelman JW, eds. Clinical Chemistry: Principles and Techniques. London: Harper and Row, 1974, 405-502.
3. Caprita R, Caprita A, Cretescu I. Estimation of Ionized Calcium and Corrected Total Calcium Concentration Based on Serum Albumin Level. *Animal Science and Biotechnologies*. 2013; 46:180-184.
4. Sava L, Sandhya P, Umesh M, Alka S. Serum calcium measurement: total versus free (ionized) calcium. *Indian Journal of Clinical Biochemistry*. 2005; 20:158-161.
5. Caprita A, Caprita R. A comparative study on the plasma ionized calcium in different animal species, Macro and Trace Elements. *Mengen und spurenelemente*. 2004; 22:360-365.
6. Doumas BT, Bayse DD, Carter RJ, Peters T, Schaffer R. A candidate reference method for determination of total protein in serum. 1. Development and validation. *Clinical Chemistry*. 1981; 27:1642-50
7. Patricia AS, Dennis J. Prediction of serum ionized calcium concentration by serum total calcium measurement in cats. *Canadian Journal of Veterinary Research*. 2010; 74:209-213.
8. Claire RS, Marie EK, Mann FA. A comparison of total calcium, corrected calcium, and ionized calcium concentrations as indicators of calcium homeostasis among hypoalbuminemic dogs requiring intensive care. *Journal of Veterinary Emergency and Critical Care*. 2009; 19:571-578.
9. Lena J, Aysha HK, Saba A. Ionized Calcium Measurement in Serum and Plasma by Ion Selective Electrodes: Comparison of Measured and Calculated Parameters. *Indian Journal of Clinical Biochemistry*. 2014; 29:327-332.
10. Nusrat A, Warda H, Aneela A, Farzana Y, Toqeer B, Umair A, Asim M. Comparison of Ionized Calcium and Albumin Corrected Total Calcium Concentration in Renal Failure Patients at Shalamar Hospital Lahore. *Pakistan Journal of Medical and Health Sciences*. 2015; 9:1-4.
11. George NB. Jr., Catherine, Brassard, Salvador FS. Measurement of ionized calcium in serum with ion selective electrodes: A mature technology that can meet the daily service needs. *Clin. Chem*. 1986; 32:1437-47.