Estimation of Ionized Calcium and Corrected Total Calcium Concentration Based on Serum Albumin Level

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Abstract

Ionized calcium is the physiologically active fraction of serum calcium and therefore its evaluation is an important clinical parameter both in mammals and birds. In the present study, concentration of total calcium (tCa), ionized calcium (iCa) based on the serum albumin level and on the total protein content, and corrected total calcium (ctCa) based on the serum albumin level were evaluated, and the correlation between these biochemical parameters was investigated in broiler chickens. The average serum iCa was 4.91±0.49g/dL representing 51.76±0.53% from the ctCa. The correlation coefficient between tCa and albumin (r = 0.8608) was greater than that between tCa and total protein (r = 0.7997). Our study illustrated that iCa and ctCa concentrations calculated from tCa and albumin are better indicators of calcium homeostasis than tCa concentrations alone.

Keywords: albumin, corrected total calcium, ionized calcium, total calcium

1. Introduction

Calcium is a very important macro element for the animal organism. Total calcium is absorbed and released by the intestines, bones, and kidneys and is biologically regulated by hormones and vitamins. The calcium ion is an essential structural component of the skeleton and plays a key role in muscle contraction, blood coagulation, enzyme activity, neural excitability, secondary messengers, hormone release, and membrane permeability. Precise control of calcium ion in extracellular fluids is vital to health. Three major hormones (PTH, vitamin D, and calcitonin) interact to maintain a constant concentration of calcium, despite variations in intake and excretion. Other hormones, such as adrenal corticosteroids, estrogens, thyroxine, somatotropin, and glucagon, may also contribute to the maintenance of calcium homeostasis.

Plasma Ca level depends on the exogenous intake (feed), resorption, excretion and the functional state of the kidneys. Calcium in plasma or serum exists in three forms or fractions: 1) ionized or free calcium, 2) calcium bound to proteins (primarily albumin), and 3) complexed or chelated calcium, bound to a variety of anions with small molecular weight (phosphate, bicarbonate, sulfate, citrate, and lactate) [1-3]. Together, the ionized and complexed calcium constitute the diffusible fraction of calcium. This portion may also be called the ultrafilterable calcium, since it passes through biologic membranes.

Ionized calcium is the physiologically active fraction of serum calcium, with important biological roles in bone homeostasis, facilitates the transmission of the nerve impulse, couples the excitation with the muscle contraction, interferes in the blood clotting and control of hormone

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secretions such as vitamin D₃ and parathyroid hormone, reduces the membrane permeability, activates metabolic and digestive enzymes, influences the utilization of iron [4,5]. About 90% of the protein-bound calcium is linked to albumin with the remaining 10% bound to a variety of globulins. Because approximately half of the calcium is protein bound, the interpretation of total calcium depends on the values for serum albumin and total protein [6]. Changes in pH alter the fraction of calcium that is bound to albumin, so the level of ionized calcium can change without alteration of total calcium. To date, many equations have been proposed to determine ionized calcium concentrations [6-13]. Some involve the albumin’s level, while others use total protein. These equations are essentially empirical equations based on a correlation between ionized calcium concentration obtained by the electrode method and an observed concentration for albumin or another substance [14].

While the measurement of total calcium, albumin and total protein is available in standard laboratories, measurement of ionized calcium remains more difficult and is generally performed only in reference laboratories. In addition, great care must be taken with sample handling. After a blood sample is collected, the ionized calcium concentration changes even more than the total calcium concentration [15-18]. Samples should be drawn anaerobically (to minimize loss of carbon dioxide), transported on ice and processed within hours (to minimize lactate generation). Heparin contamination must be avoided as it interferes with the assay. These stringent conditions make accurate measurement of ionized calcium problematic in many settings. Total calcium measurements are relatively cheap, readily available, and more resistant to sample transportation variables. Laboratories routinely determine the total calcium concentration which measures all 3 forms of calcium. Corrected calcium concentration estimates the total concentration as if the albumin concentration was normal. Clinical laboratories use several equations to calculate corrected calcium values. Some involve the albumin level, while others use total protein.

In the present study, concentration of total calcium (tCa), ionized calcium (iCa) based on the serum albumin level and on the total protein content, and corrected total calcium (ctCa) based on the serum albumin level were evaluated, and the correlation between these biochemical parameters was investigated in broiler chickens.

2. Materials and methods

The study was made on blood samples from broiler chickens. Serum samples have been collected at 42 day of age for the estimation of total protein, albumin, and calcium. The serum total protein (TP) was determined with the biuret reagent method as described by Cannon (1974) [19], using a PerkinElmer UV/VIS-Lambda35 spectrophotometer. The serum albumin concentration was determined using modified bromocresol green colorimetric method as described by Doumas et al. (1971) [20]. Determination of tCa concentration in serum was carried out using atomic absorption spectroscopy (AAS). Ionized calcium and corrected total calcium were calculated (in mg/dL) with the formula [21]:

\[
iCa = [0.9 + (0.55 \times tCa – 0.3 \times albumin)]
\]

\[
iCa = (6 \times tCa – TP)/3)/(6 + TP)
\]

\[
ctCa = tCa – 0.707 \times (albumin – 3.4),
\]

where tCa is in mg/dL, albumin and TP are in g/dL.

Serum components were expressed as means and standard deviation, which were submitted to statistical analysis.

3. Results and discussion

The obtained experimental data are presented in Table 1. The average ctCa, calculated using tCa and albumin, was 9.47±0.87 mg/dL, with 18.07% greater than tCa determined by AAS. Serum iCa calculated from tCa and TP [22] resulted in higher values than iCa calculated from tCa and albumin with 10.20%. Serum iCa calculated from tCa and albumin ranged from 3.96 mg/dL to 5.56 mg/dL with an average of 4.91±0.49g/dL. Serum iCa calculated from tCa and TP ranged from 4.12 mg/dL to 6.34 mg/dL with an average of 5.46±0.62g/dL.
Although there was a very high positive correlation ($r = 0.9884$) between iCa calculated with both equations (Figure 1), the values based on albumin concentration are the most suitable, since 80-90% of protein bound calcium is an albumin chelate [23].

Values close to normal were obtained when calculating the percentage of iCa from ctCa (51.76±0.53%) than when calculating percentage of iCa from tCa (61.40±1.83%).

The correlation coefficient between tCa and albumin ($r = 0.8608$) (Figure 2) was greater than that between tCa and total protein ($r = 0.7997$) (Figure 3).

Physiologically important changes in iCa can be produced without change in the tCa concentration by altering the affinity of albumin for calcium. Measurement of total serum calcium, particularly if corrected for the serum albumin, is usually adequate for most situations.

### Table 1. Determined and calculated biochemical parameters in avian serum

<table>
<thead>
<tr>
<th>Sample</th>
<th>tCa (mg/dL)</th>
<th>TP (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>iCa (TP)$^1$ (mg/dL)</th>
<th>iCa (A)$^2$ (mg/dL)</th>
<th>ctCa (mg/dL)</th>
<th>%iCa from ctCa$^3$</th>
<th>%iCa from tCa$^3$</th>
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Xm±DS 8.02±1.02 2.63±0.18 1.34±0.25 5.46±0.62 4.91±0.49 9.47±0.87 51.76±0.53 61.40±1.83

$^1$iCa (TP) = iCa calculated from tCa and total protein

$^2$iCa (A) = iCa calculated from tCa and albumin

$^3$calculated based on iCa (A)
4. Conclusions

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 iCa and ctCa concentrations calculated from tCa and albumin are better indicators of calcium homeostasis than tCa concentrations alone.

References


