

# Adjusted calcium concentration as a predictor of ionized hypocalcemia in hypoalbuminemic dogs

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Email: fiammagdw@gmail.com**Abstract**

**Background:** Ionized calcium (iCa) is the biologically active fraction of total calcium (tCa) with clinical relevance to evaluate calcium homeostasis, but not all primary veterinarians have access to serum iCa. Formulas that adjust tCa to correct for variability in serum protein concentrations were not designed to predict iCa and are considered unreliable surrogates for iCa.

**Objectives:** To determine whether adjusted calcium concentration (aCa) can predict ionized hypocalcemia in hypoalbuminemic dogs without hyperphosphatemia.

**Animals:** A total of 262 hypoalbuminemic dogs without hyperphosphatemia.

**Methods:** Retrospective review of paired tCa and iCa. Patients were included if serum albumin concentration was  $\leq 2.5$  g/L and serum phosphorus concentration was  $\leq 5$  mg/dL. The aCa was calculated using  $tCa$  (mg/dL) – serum albumin concentration (g/dL) + 3.5 (g/dL). Sensitivity, specificity, positive (PPVs) and negative (NPVs) predictive values, and accuracy were determined for tCa and aCa at predicting any ( $< 1.13$  mmol/L) and moderate ( $< 1.02$  mmol/L) ionized hypocalcemia. Patients also were stratified into mild-to-moderate (2.0–2.5 g/dL) and severe hypoalbuminemia ( $< 2.0$  g/dL).

**Results:** A total of 4296 dogs had paired results of which 262 met the inclusion criteria. Of these, 35 (13.4%) dogs had iCa  $< 1.13$  mmol/L and 13 dogs (5.0%) had concentrations  $< 1.02$  mmol/L. The sensitivity, specificity, NPVs and PPVs of a decreased tCa and aCa for detecting moderate ionized hypocalcemia were 100% and 92.3%, 57.8% and 94.8%, 100% and 99.6%, and 11.0% and 48.2%, respectively, and accuracy was 60.0% and 94.7%, respectively.

**Conclusions:** A low aCa was useful to detect ionized hypocalcemia in hypoalbuminemic nonhyperphosphatemic dogs. A normal aCa indicated that moderate ionized hypocalcemia was unlikely.

**KEYWORDS**

canine, ionized calcium, protein-losing enteropathy, total calcium

**Abbreviations:** aCa, adjusted calcium concentration; AUC, area under the curve; CI, confidence interval; iCa, ionized calcium concentration; NPVs, negative predictive values; PLEs, protein-losing enteropathies; PPVs, positive predictive values; ROCs, receiver operating curves; tCa, total calcium concentration.

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## 1 | INTRODUCTION

Serum total calcium (tCa) exists in 3 fractions: protein-bound (mostly to albumin), ionized calcium (iCa), and complexed calcium. In clinically normal dogs, these fractions account for approximately 55%, 35%, and 10%, respectively.<sup>1</sup> Ionized calcium (iCa) and complexed calcium are the biologically active fractions. However, only tCa and iCa can be measured in veterinary clinics. Ionized calcium is the most clinically relevant fraction to evaluate the calcium status.<sup>2</sup> Although iCa is not determined by routine chemistry analyzers, it can be readily measured on portable analyzers. Suitable point-of-care analyzers have not yet been universally adopted by veterinary clinics and tCa, as a component of most serum biochemistry panels, remains common to measure and report serum calcium concentration in dogs and cats. Changes in proteins or anions, such as in patients with renal disease and hyperphosphatemia,<sup>3</sup> affect the complexed portion of calcium, and hypoalbuminemic states affect the protein-bound calcium fraction.<sup>4</sup> Ionized calcium concentration is influenced by pH and blood gas status in vivo and in vitro.<sup>2</sup> Acidosis will shift some calcium to the ionized fraction and alkalosis will shift some calcium away from the ionized fraction.<sup>2</sup>

Formulas to adjust tCa to correct for serum protein concentration have been evaluated in veterinary medicine.<sup>4-6</sup> Previous studies have shown that, as albumin or total protein concentrations decrease, so does the tCa in a linear fashion.<sup>5</sup> These adjustment formulas were not designed to predict iCa and a subsequent study showed that the use of these formulas to calculate adjusted calcium (aCa) by correcting serum tCa for the total protein or albumin concentration was unreliable surrogates for iCa.<sup>4</sup> This may in part be because such studies consisted of a heterogeneous population, including many patients with renal disease and hyperphosphatemia, and the formulas used do not take into account the complexed fraction of calcium, but assume that iCa and serum albumin concentrations are the sole determinants of tCa concentration.<sup>3,4</sup>

Although formulas to adjust calcium concentrations are not helpful when applied to a general population, they still might be helpful in patients with hypoalbuminemia.

Formulas to adjust tCa for decreased albumin (or total protein) concentration are useful to determine if the decrease in tCa is due to changes in serum albumin concentration, but they are not designed for and do not predict the concentration of iCa in the general population.<sup>5,6</sup> However, perhaps the formulas could be used to predict the presence or absence of decreased iCa in a specific clinical situation in which patients have hypoalbuminemia but not hyperphosphatemia, where the assumption that iCa and serum albumin concentration as the primary determinants of Ca concentration is more valid.

An important clinical scenario associated with ionized hypocalcemia is patients with protein-losing enteropathies (PLEs).<sup>7</sup> Hypoalbuminemia and ionized hypocalcemia are common in these patients,<sup>8,9</sup> but derangements in serum phosphorus concentration are less common. The confounding effect of hypoalbuminemia on the accurate assessment of calcium status is important in dogs with chronic enteropathies, because they have lower serum concentrations

of 25-hydroxyvitamin D and it is associated with a negative outcome.<sup>1,7,10-13</sup> Failure to accurately assess ionized calcium homeostasis in such patients might result in suboptimal management of acute or chronic ionized hypocalcemia and additional tools to identify patients at risk for complications of ionized hypocalcemia could be useful to veterinary practitioners who are not able to immediately measure iCa.

Our aim was to determine whether aCa could be predictive of the presence or absence of ionized hypocalcemia in a population of hypoalbuminemic dogs without hyperphosphatemia. Our hypothesis was that aCa could be a clinically useful predictor of ionized hypocalcemia in hypoalbuminemic, nonhyperphosphatemic dogs.

## 2 | MATERIALS AND METHODS

Laboratory records from June 22, 2014 to February 23, 2018 from 2 sites of 1 veterinary referral center were retrospectively searched. Records were retrieved where a biochemistry panel (including a minimum of albumin, tCa, and phosphorus concentrations) and an iCa were available for the same dog and had been measured within 24 hours of each other. The same models of laboratory analyzer were used at both locations and were calibrated and maintained according to manufacturer recommendations. Serum was used for measurement of tCa (DRI-CHEM 7000, Fujifilm, Heska, Loveland, Colorado), and lithium heparinized whole blood were used for measurement of iCa (Element-POC, Siemens, Heska, Loveland, Colorado). The samples for iCa were handled anaerobically and with the recommended ratio of heparin to blood. Samples for iCa were not corrected for pH. During the study period, there were no changes in the methodologies or reagents used by the laboratory analyzers. Samples submitted to reference laboratories were excluded. If multiple pairs of calcium measurements were available from the same dog, only the first pair was included in the analysis and subsequent results for that dog were excluded.

Adjusted Ca (aCa) was calculated using the previously published formula:  $aCa = tCa \text{ (mg/dL)} - \text{albumin (g/dL)} + 3.5 \text{ (g/dL)}$ . This formula was developed and tested in over 3000 dogs using linear regression and was similar to that originally developed for people in the 1970s.<sup>12,14,15</sup> Dogs were included in the study group if serum albumin concentration was  $\leq 2.5 \text{ g/L}$  (reference range, 2.5-4 g/dL) and serum phosphorus concentration  $\leq 5 \text{ mg/dL}$  (reference range, 1.9-5 mg/dL). Two groups of dogs were studied based on the severity of ionized hypocalcemia: any ionized hypocalcemia ( $< 1.13 \text{ mmol/L}$ ) and moderate ionized hypocalcemia (iCa  $< 1.02 \text{ mmol/L}$ ). The moderate ionized hypocalcemia cutoff was arbitrarily chosen as 10% below the lower end of the reference range (1.13-1.42 mmol/L) for clinical relevance.<sup>6</sup>

### 2.1 | Statistical analysis

Data were visually inspected for normality and presented as median (range). For each group, receiver operating curves (ROCs)

were constructed and the area under the curve (AUC) was calculated for tCa and aCa to predict each of 2 levels of ionized hypocalcemia.

Sensitivity (the proportion of positives that are correctly identified), specificity (the proportion of negatives that are correctly identified), positive predictive value (PPV, the probability that a positive result is truly positive) and negative predictive value (NPV, the probability that a negative result is truly negative), and accuracy (defined as the proportion of aCa or tCa results that correctly predicted ionized hypocalcemia) were calculated for tCa and aCa to predict the 2 levels of ionized hypocalcemia defined above using the lower end of the laboratory reference range for tCa of 9 mg/dL (reference range, 9-12.2 mg/dL) as the cutoff for both tCa and aCa.

The Youden method helps to determine the performance of a diagnostic test and was used to determine the optimal cutoff for tCa and aCa to establish each level of ionized hypocalcemia. Sensitivity, specificity, PPV, NPV, and accuracy then were calculated for the optimal cutoff for tCa and aCa determined by the Youden method.

Finally, the patients were stratified into mild-to-moderate hypoalbuminemia, defined as serum albumin concentration between 2.0 and 2.5 g/dL, and severe hypoalbuminemia, defined as serum albumin concentration < 2.0 g/dL. The Youden method again was used to determine specific cutoffs for tCa and aCa for each subgroup, and sensitivity, specificity, PPV, NPV, and accuracy also were recalculated using the chosen cutoffs.

All statistical analyses were carried out using an open access software program (A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria. 2019. <https://www.R-project.org/>).

### 3 | RESULTS

Paired results measured within 24 hours of each other were available for 4296 dogs of which 528 (12.3%) had serum albumin concentration  $\leq$  2.5 g/L, 2960 (68.9%) had a serum phosphorus concentration  $\leq$  5 mg/dL, and 262 (6.1%) had both an albumin  $\leq$  2.5 g/L and phosphorus concentration  $\leq$  5 mg/dL.

The median time difference between measurement of iCa and tCa was 56 minutes (range, 0-1334 minutes). In 160 (60%) patients, the iCa was measured first (with a median delay of 50 minutes [range, 0-1334 minutes] until measurement of tCa), and in 102 (40%) patients the tCa was measured first (with a median delay of 58 minutes [range, 0-1296 minutes] until measurement of iCa).

The median iCa was 1.25 mmol/L (range, 0.7-1.51 mmol/L). Thirty-five (13.4%; 95% confidence interval [CI], 9.8%-18.0%) had an iCa of <1.13 mmol/L and 13 (5.0%; 95% CI, 2.5%-8.3%) had an iCa of  $\leq$ 1.02 mmol/L and 40.5% had a tCa of <9 mg/dL. Nine dogs (3.6%) had ionized hypercalcemia (>1.42 mmol/L). Of the 528 dogs with hypoalbuminemia, 191 (36.2%) had a tCa <9 mg/dL (the lowest tCa was 3.3 mg/dL), 93 had an iCa <1.13 mmol/L (17.6%), and 66 (12.5%) had both total and ionized hypocalcemia. Of the 262 dogs

with both hypoalbuminemia and normal serum phosphorus concentration, 100 (40.5%) had a low tCa (<9 mg/dL), 76 (29%) had a low tCa and normal iCa, and 30 (11.5%) had both total and ionized hypocalcemia.

#### 3.1 | Accuracy of tCa and aCa to predict ionized hypocalcemia

The AUC for the ROC for the identification of moderate ionized hypocalcemia was similar for both tCa (AUC, 0.98; 95% CI, 0.95-1.0) and aCa (AUC, 0.99; 95% CI, 0.97-1.0). The sensitivity and specificity of tCa for detecting any ionized hypocalcemia (iCa <1.13 mmol/L) were 93.3% and 61.2%, respectively. The sensitivity and specificity for identifying moderate ionized hypocalcemia (iCa  $\leq$ 1.02 mmol/L) were 100% and 57.8%, respectively (Table 1). The sensitivity and specificity for aCa were 51.4% and 96.9%, respectively, for detecting any ionized hypocalcemia, and 92.3% and 94.8%, respectively, for detecting moderate ionized hypocalcemia (Figure 1).

For low tCa and aCa to predict a moderate ionized hypocalcemia, the PPV was 11.0% and 48.2% and the NPV was 100.0% and 99.6%, respectively. Accuracy was defined as the percentage of cases that correctly predicted if any ionized hypocalcemia or moderate ionized hypocalcemia was present. When a cutoff of tCa of 9.0 mg/dL was used to detect moderate ionized hypocalcemia, the accuracy for aCa was 94.7% and tCa was 60.0% (Table 1). The accuracies for aCa and tCa to detect iCa below the reference range or moderate ionized hypocalcemia are presented in Table 1.

#### 3.2 | Optimal thresholds to predict ionized hypocalcemia

Using the Youden method to determine an optimal threshold, a cutoff for tCa of 8.3 mg/dL was found to identify any ionized hypocalcemia (iCa <1.13 mmol/L) and a tCa cutoff of 7.0 mg/dL was optimal for the identification of moderate ionized hypocalcemia (iCa  $\leq$ 1.02 mmol/L).

The sensitivity, specificity, NPV, PPV, and accuracy at these cutoffs are shown in Table 1. We calculated the ideal cutoff to detect any ionized hypocalcemia (iCa < 1.13 mmol/L) and moderate ionized hypocalcemia (iCa < 1.02 mmol/L). The ideal cutoffs of tCa and aCa to detect any ionized hypocalcemia were 8.3 mg/dL and 9.9 mg/dL, respectively, both quite different from the tCa cutoff of 9.0 mg/dL provided by the laboratory. However, the ideal cutoffs of tCa and aCa to detect moderate ionized hypocalcemia were 7.0 mg/dL and 9.4 mg/dL, respectively. The sensitivity, specificity, NPV, and PPV of a decreased tCa and aCa for detecting moderate ionized hypocalcemia were 100% and 92.3% (95% CI, 76.9%-100.0%), 57.8% (95% CI, 50.4%-63.9%) and 94.8% (95% CI, 91.6%-97.2%), 100% and 99.6% (95% CI, 98.7%-100.0%), and 11.0% (95% CI, 9.7%-12.6%) and 48.2% (95% CI, 35.7%-65.0%), respectively. Accuracy for aCa was 94.7% (95% CI, 91.6%-97.3%) and 60.0% (95% CI, 53.8%-65.7%) for tCa to detect moderate ionized hypocalcemia.

**TABLE 1** Sensitivity, specificity, NPV, PPV, and accuracy (95% confidence interval) of different cutoffs for total calcium (tCa) and adjusted calcium (aCa) to identify two levels of decreased ionized calcium (iCa) (ionized hypocalcemia) in 262 hypoalbuminemic and nonhyperphosphatemic dogs. Rows with bold values use the 9.0 mg/dL cutoff provided by the laboratory for tCa, rows with unbold values use the optimal cutoff determined by the Youden method

Concentration of iCa (mmol/L)	Cutoff		Sensitivity %		Specificity %		NPV %		PPV %		Accuracy %	
	tCa (mg/dL)	aCa	tCa	aCa	tCa	aCa	tCa	aCa	tCa	aCa	tCa	aCa
<1.13 (n = 35)	9.0		93.3 (83.3-100)	51.4 (34.3-68.6)	61.2 (54.8-67.6)	96.9 (94.3-99.1)	98.6 (96.4-100)	24.8 (21.6-28.6)	72.4 (55.2-88.0)	65.1 (59.4-70.7)	90.8 (87.8-94.0)	
	8.3	9.9	82.9 (65.7-94.3)	85.7 (68.6-97.1)	85.0 (76.7-95.6)	84.1 (75.3-95.2)	97.0 (94.6-99.0)	45.9 (35.7-71.8)	45.6 (35.2-71.1)	84.7 (77.9-92.4)	84.4 (76.7-92.8)	
<1.02 (n = 13)	9.0		100.0 (100.0-100.0)	92.3 (76.9-100.0)	57.8 (50.4-63.9)	94.8 (91.6-97.2)	100.0 (100.0-100.0)	11.0 (9.7-12.6)	48.2 (35.7-65.0)	60.0 (53.8-65.7)	94.7 (91.6-97.3)	
	7.0	9.4	100.0 (84.6-100.0)	100.0 (100.0-100.0)	96.0 (80.3-98.8)	93.6 (90.0-98.4)	100 (99.2-100)	54.6 (21.0-81.3)	44.8 (34.2-76.5)	95.8 (81.3-98.9)	93.9 (90.5-98.5)	

### 3.3 | Impact of severity of hypoalbuminemia on optimal cutoffs for tCa and aCa

To further investigate the optimal cutoff for tCa, the dogs were stratified into those with mild-to-moderate hypoalbuminemia (197 dogs) and those with severe hypoalbuminemia (65 dogs). For dogs with mild-to-moderate hypoalbuminemia, a cutoff for tCa of 8.5 mg/dL was optimal to identify any ionized hypocalcemia. A cutoff of 8.2 mg/dL was optimal for the identification of moderate ionized hypocalcemia.

For dogs with severe hypoalbuminemia, a cutoff for tCa of 7.6 mg/dL was derived to identify any hypocalcemia. A cutoff of 7.0 mg/dL for tCa was optimal for the identification of moderate ionized hypocalcemia. Optimal cutoffs and corresponding sensitivity, specificity, NPV, PPV, and accuracy are shown in Table 2.

## 4 | DISCUSSION

Our aim was to determine whether aCa could be predictive of any moderate ionized hypocalcemia in a population of hypoalbuminemic dogs without hyperphosphatemia. Using the laboratory reference range for tCa, the PPV and overall accuracy of aCa was superior to that for tCa.

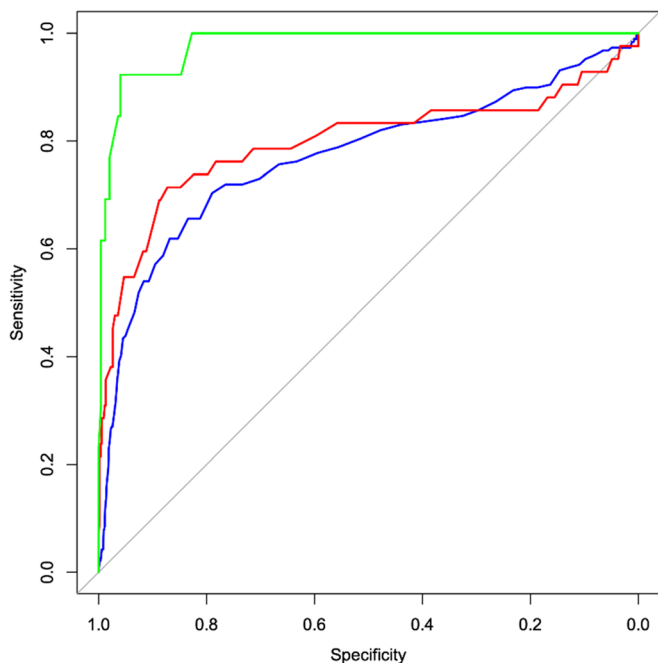
In hypoproteinemic states, tCa is not a reliable indicator of iCa because hypoalbuminemia causes a decrease in the protein-bound fraction of the calcium.<sup>2</sup> This was shown in the regression analysis of previous studies.<sup>5,6</sup>

Previous studies showed that aCa formulas can be used when serum total protein or albumin concentration is decreased to predict that the decrease in tCa is due to the decrease in serum protein concentration.<sup>5,6</sup> However, neither of those studies tried to use the aCa formula to predict the iCa or a decrease in the iCa. Consequently, direct measurement of iCa has been the method endorsed by most investigators if iCa is needed.<sup>4-6</sup>

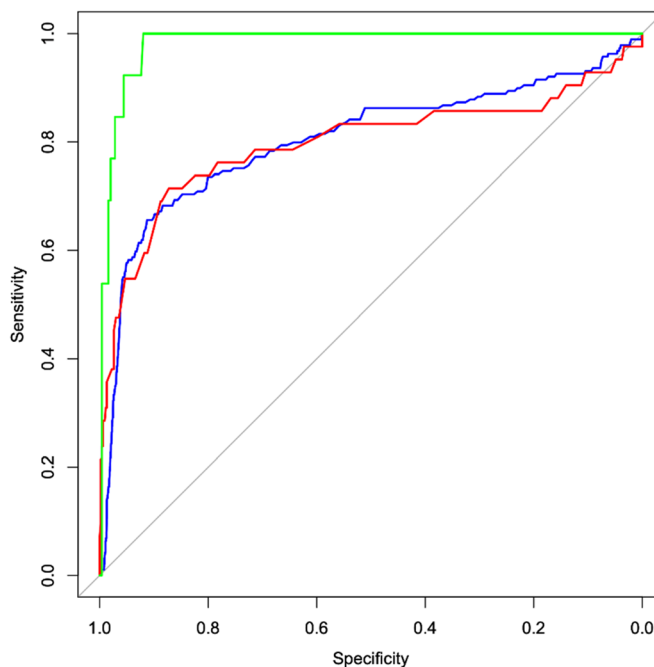
Our study indicates that, in hypoalbuminemic dogs without hyperphosphatemia, the previously published formula to calculate the aCa can be used. Because hypoalbuminemia is the most common cause of a low serum tCa in dogs,<sup>4</sup> the aCa can be used to accurately predict ionized hypocalcemia and help clinicians make treatment decisions, particularly when an iCa analyzer is not immediately available. In addition, it also helps to determine when iCa should be measured.

A previous study of a heterogeneous population of sick dogs showed that the accuracy of the aCa to predict iCa was poor (63%), and worse than that for tCa (accuracy of 73%).<sup>4</sup> This population included dogs that were hypercalcemic, normocalcemic, and hypocalcemic. Included within the heterogeneous population of sick dogs in that study were 30% with chronic kidney disease, and aCa had a particularly poor accuracy in this subpopulation (47% accuracy).<sup>4</sup> This may have been because of increases in complexed calcium because phosphorus is an important complexer of calcium.<sup>4</sup> In a subgroup of 1143 sick dogs with diseases other than chronic renal disease, the reported accuracy and specificity of the aCa were comparable to our

(A) : Graph for tCa



(B) graph: Graph for cCa



**FIGURE 1** Receiver operating characteristic curve for tCa and aCa. Three populations: in green the hypoalbuminemic nonhyperphosphatemic (n = 262) dogs; in red the hypoalbuminemic (any phosphorus status) (n = 576) dogs, and in blue all the cases (n = 4296)

**TABLE 2** Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy (95% confidence interval) of total calcium at different cutoffs (determined by Youden method) to identify 2 levels of decreased ionized calcium (iCa) (ionized hypocalcemia) in 2 groups of patients stratified for level of hypoalbuminemia

Albumin level (g/dL)	To identify a iCa of	tCa threshold (mg/dL)	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)	Accuracy % (95% CI)
2-2.5 (n = 197)	<1.13	8.5	66.7 (46.7-93.3)	91.2 (70.9-97.3)	97.2 (95.3-99.4)	39.3 (17.2-66.7)	89.3 (71.6-94.9)
	<1.02	8.2	100.0 (100.0-100.0)	94.9 (90.7-99.5)	100.0 (100.0-100.0)	23.1 (14.3-75.0)	94.9 (90.9-99.5)
<2.0 (n = 65)	<1.13	7.6	91.1 (65.0-100.0)	91.1 (60.0-100.0)	93.2 (85.7-100.0)	79.0 (52.6-100.0)	87.7 (72.3-95.4)
	<1.02	7.0	100.0 (90.0-100.0)	94.6 (85.5-100.0)	100.0 (98.2-100.0)	76.9 (55.6-100.0)	95.4 (87.7-100.0)

Abbreviations: CI, confidence interval; tCa, total calcium.

results.<sup>4</sup> However, previous studies did not specifically investigate hypoalbuminemic dogs without hyperphosphatemia. Although our study included only 262 dogs of 4296, it is still a clinically relevant population; it encompasses many patients with PLE that are prone to hypocalcemia and the associated complications.<sup>12,13</sup> Hyperphosphatemia, rather than azotemia, was used as an exclusion criterion because chronic renal failure usually is accompanied by hyperphosphatemia because of decreased glomerular filtration of the phosphate load.<sup>2,4</sup>

In our population of hypoalbuminemic, nonhyperphosphatemic dogs, the AUCs for aCa and tCa for predicting ionized hypocalcemia were very similar. However, when using the laboratory reference range for tCa, aCa had a higher specificity, PPV, and accuracy compared to tCa for identifying hypocalcemia. This apparent discrepancy occurs because ROC analysis assesses the performance of a test across a range of potential cutoffs. In this case, the optimal cutoff for

aCa in hypoalbuminemic dogs was close to the laboratory reference range for tCa whereas the optimal cutoff for tCa was substantially lower than the reference range. The sensitivity of aCa for identifying any ionized hypocalcemia (<1.13 mmol/L) was only 53.3% and accurate determination of the actual iCa always requires measurement of iCa in the blood.

However, our study made a dichotomous assessment of the presence or absence of hypocalcemia as an initial screening test and the presence of very mild hypocalcemia is unlikely to be clinically relevant for acute case management. For this reason, we also investigated the utility of aCa for predicting the presence of an iCa <1.02 mmol. Although this was an arbitrary reflection of biochemical severity and the association with clinical signs was not assessed in our study, we defined it as “moderate ionized hypocalcemia” because this severity of ionized hypocalcemia is likely to prompt further investigation or treatment of the ionized hypocalcemia.<sup>2,16</sup>

The sensitivity of aCa and tCa to predict moderate ionized hypocalcemia was 100%. Additionally, because the high specificity for predicting both any and a moderate ionized hypocalcemia, dogs with a low calculated aCa are likely to have at least a moderate ionized hypocalcemia.

In our study, when any ionized hypocalcemia was present, tCa was a better screening test than aCa because tCa has higher sensitivity. However, as hypocalcemia worsens and it becomes more important to correctly identify decreased iCa, the sensitivity of tCa and aCa is similar once there is a moderate decrease in tCa (hypocalcemia).

The advantage of aCa comes when considering the specificity, accuracy, and PPV using the lower laboratory reference range for tCa of 9.0 mg/dL. Regardless of the severity of ionized hypocalcemia, these values are higher for aCa than tCa, which means that aCa will reflect the patients with ionized hypocalcemia once hypoalbuminemia has been taken in consideration. The fact that the specificity is low for tCa but the NPV is high reflects that a low tCa is likely to be due to other causes than ionized hypocalcemia, such as hypoalbuminemia, in this population of dogs.<sup>5,6</sup>

This finding gives aCa potential utility as an initial screening test, because although some dogs with ionized hypocalcemia may be missed, most with at least moderate ionized hypocalcemia that could be contributing to clinical signs or might require clinical intervention will be correctly identified.

When investigating dogs with at least moderate ionized hypocalcemia (iCa <1.02 mmol/L), specificity was 95.0% and PPV was 47.8% with an overall accuracy of 95.2%. Although the low prevalence of moderate ionized hypocalcemia led to a relatively low PPV, it was still more than 3 times higher than the corresponding tCa. False identification of hypocalcemia leading to additional screening or treatment is likely to be less clinically deleterious than overlooking patients with moderate ionized hypocalcemia.<sup>16</sup>

Several formulas for aCa exist, with some adjusting for total protein concentration and others for serum albumin concentration.<sup>5,6</sup> In our study, the most commonly used formula in veterinary medicine was used, based on the formula first proposed for human patients in 1973.<sup>14,15</sup> Previous studies have used this formula to predict total hypocalcemia caused by a decrease in serum total protein or albumin concentration.<sup>5,6</sup> If hypocalcemia is caused by concurrent hypoalbuminemia, causes of hypoalbuminemia should be investigated. On the contrary, if hypocalcemia cannot be attributed to hypoalbuminemia, then further investigation is required to identify diseases that cause hypocalcemia. The formulas previously developed<sup>5,6</sup> are useful to predict if a decrease in tCa is caused by hypoproteinemia or hypoalbuminemia, and they were not intended to and do not predict the iCa. Both studies had similar results, namely, that as the severity of hypoproteinemia progresses, especially hypoalbuminemia, the greater the decrease in serum tCa. Our results support these observations. When we stratified our results by serum albumin concentration, we noted that the accuracy and PPV of tCa to predict ionized hypocalcemia deteriorated. This is expected because the decrease in tCa was due to the decrease in serum albumin concentration not to a decrease in iCa.

Our study confirms that hypoalbuminemia decreases the tCa in a larger percentage of the population (40.5%), but only a smaller percentage (13.4%) will have decreases in both in tCa and iCa. However, previous studies did not attempt to predict ionized hypocalcemia as we did and this approach is a novel use of the formula for this particular population of dogs with hypoalbuminemia but not hyperphosphatemia.<sup>5,6</sup>

It was beyond the scope of our study to compare the clinical utility of different published formulas.

The aCa is calculated number that helps us interpret clinicopathologic data for our patients. The important concentrations of calcium in the blood are the measured tCa or iCa (and the complexed calcium, although this is not commonly measured).<sup>1,2</sup>

Although aCa performed much better than tCa for identifying hypocalcemic patients in this group, the initial ROC analysis showed similar AUCs. This finding suggests that the intrinsic discriminatory capacity of tCa and aCa is similar and the difference in performance is because the laboratory cutoffs are not appropriate for tCa in hypoalbuminemic patients. Therefore, an alternative to calculating the aCa in hypoalbuminemic and nonhyperphosphatemic patients would be to use the unadjusted tCa with an amended cutoff to optimize its sensitivity and specificity for predicting ionized hypocalcemia in this population.

The accuracy of tCa could be further improved by using cutoffs stratified by severity of hypoalbuminemia. Although these approaches result in equally valid interpretations of the laboratory parameters, recalling different ranges for different albumin levels is less convenient for clinicians than using a universal cutoff for aCa and tCa.

Our study had some limitations. The study was retrospective in nature and based on clinicopathologic data, without regard to dog signalment, clinical signs, other laboratory abnormalities, or final diagnosis. Although these factors would not impact the conclusion of our study, the association between aCa and iCa could vary based on disease state. Treatments affecting calcium concentration might have been given between paired tCa and iCa concentration determinations. However, although paired samples for tCa and iCa were obtained within 24 hours, the median delay between both was only 56 minutes, minimizing the chances that any treatment affecting the calcium concentration might be given between obtaining both samples. Furthermore, if any treatment for hypocalcemia occurred in between sample collection, the data would tend to underestimate the strength of the relationship between tCa or aCa and iCa. Furthermore, blood samples were not screened for hemolysis, icterus, acidosis, or alkalosis, and these factors can alter the measured tCa and iCa in various ways.<sup>2</sup> Finally, the predictive values from our study must be extrapolated with caution because the prevalence of ionized hypocalcemia may vary among dog populations.

## 5 | CONCLUSION

In this population of hypoalbuminemic nonhyperphosphatemic dogs, aCa can be used as a screening test for ionized hypocalcemia. The

PPV of a low aCa to predict a moderate hypocalcemia (iCa  $\leq$ 1.02 mmol/L) was 3 times higher than the PPV of tCa and the overall accuracy also was higher at 95.2% compared to 59.9% accuracy for tCa.

Measured iCa in blood (serum, plasma) remains the gold standard for determination of the biologically active blood calcium fraction on which to base treatment decisions in critically ill patients and in patients with ionized hypocalcemia.

However, aCa predicted the presence or absence of moderate ionized hypocalcemia with good accuracy in this population of hypoalbuminemic nonhyperphosphatemic dogs and can be a useful screening test to guide treatment decisions in this specific clinical scenario.

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#### CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

#### OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

#### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

#### HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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