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Determination of Blood Biochemistry Reference Intervals in Eastern Collared Lizards (*Crotaphytus collaris*)

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Abstract

Eastern collared lizards (*Crotaphytus collaris*) are growing in popularity as exotic pets in the United States and worldwide. However, clinical biochemistry reference data to support the interpretation of health and disease for this species are lacking. This study evaluated 87 apparently healthy eastern collared lizards (*C. collaris*). Blood samples were collected from the ventral coccygeal vein and assayed via an Abaxis VetScan VS2 analyzer and avian/reptile profile plus rotor. Although lizard weight and snout–vent length (SVL) were normally distributed, most biochemical analytes, except albumin and total protein, were not. Many analytes were affected, albeit slightly, by lizard age, SVL, body condition score, gravidity and/or recent oviposition, sex, health status, and color and locality. However, except for calcium, phosphorous, and albumin for gravid or immediately postoviposition females, biochemical values remained within the generated reference interval. Limitations of this study included that the VetScan VS2 avian/reptile profile plus rotor was unable to successfully provide values for bile acids for most of these apparently healthy lizards. When compared to biochemistry analyte values of lizards from the suborder *Iguania* from the western hemisphere, eastern collared lizard biochemistries were similar for some analytes, but a relatively increased plasma glucose and uric acid occurs in this species, which could affect the diagnosis of clinical disease or other health abnormalities.

Key Words: Blood, coccygeal vein, plasma, squamate, Sauria

Introduction

The eastern collared lizard, *Crotaphytus collaris*, ranges from the southwestern part of the United States to Mexico, eastward to Arkansas and Missouri, and as far west as Arizona and Utah (Burt, 1928; Templeton *et al.*, 2001). This species of lizard is becoming more popular in the pet trade because of its wide range of natural color variation, similar husbandry to that of the bearded dragon (*Pogona vitticeps*), and interesting behaviors. However, little is known regarding the biochemistry data of healthy animals or how sex, snout–vent length (SVL), age, or color variation affect these analytes. For eastern collared lizards, only hematological parameters from blood collected from the orbital sinus provided male, female, and total ranges. In this species, males had higher packed-cell volume and hemoglobin values than females and seasonal variations occurred in females likely associated with the seasonal reproductive cycle (Ballinger, 1985). In Dickerson's collared lizards (*Crotaphytus dickersonae*), color traits were associated with physiological performance (Plasman *et al.*, 2015). The aim of this study was to evaluate the variations of biochemistry analytes by color and locale, age, sex, reproductive status, weight, SVL, and body condition score (BCS) and to calculate specific reference intervals (RIs) to provide diagnostic guidance for veterinarians providing health care to these species. We hypothesized that biochemistry values would vary based on sex and reproductive status for calcium (Ca) and that there would be no variation in other biochemistry analytes based on lizard age, weight, SVL, or color and locality.

Materials and Methods

Animals and sample collection: Eighty-seven captive, clinically healthy eastern collared lizards were used in this study. Seventy-two eastern collared lizards, 47 males and 25 females, were captive bred from a private collection and 15 eastern collared lizards, 9 males and 6 females, were wild caught

from New Mexico by one of the authors (BJL). Collection was authorized via Special Purpose Permit: Amphibian and Reptile Commercial Collecting Permit per New Mexico regulation 19.35.10 NMAC, (Permit #90 accorded to BJL permittee). All of the collared lizards were handled and bled using methods approved by the Texas A&M Institutional Animal Care and Use Committee (TAMU-IACUC 2018-0049). Samples were collected in June, which we assessed as the late spring and early summer season. The lizards were fed 24 h before sampling. Lizards were included in the study based on apparent health and normality of physical examination, behavior, food and water intake, and negative direct fecal and flotation result before study. All lizards were maintained in similar aquarium enclosures, with similar husbandry before and during this study as recommended for the species (Louth and Heatley, 2020). For each animal, weight (g), SVL (cm), sex, BCS (1–5), age, color, and locale were determined and recorded. Females were evaluated for the presence of progesterone-induced blush of gravidity, and egg development was confirmed based on physical palpation and/or ultrasound (Cooper and Ferguson, 1973). Color and locale were identified as follows: light blue Texas (LBTX), yellow & blue New Mexico (YBNM), yellow New Mexico (YNM), yellowhead (Baileyi) (YHB), and aquaflame Oklahoma (AQFOK).

Sample handling and analysis: Via manual restraint, blood samples of 0.15–0.45 ml were obtained from the ventral coccygeal vein with a 26-G needle and 1-ml syringe, with no greater than 10% of the lizard's estimated blood volume being withdrawn (Fig. 1) (Redrobe and MacDonald, 1999). Subcutaneous fluids were given at 20 ml/kg of Plasma-Lyte (Baxter Healthcare Corporation, Deerfield, IL. USA) after sample collection for rehydration. Samples were placed in 1-ml green-top microtainer tubes (Vacuette MiniCollect®; Greiner Healthcare Solutions, Cranberry Twp, PA, USA) with lithium heparin. Within 20 min of collection, the sample was analyzed using VetScan Abaxis avian/reptilian profile plus rotor and VetScan VS2 (Abaxis North America, Union City, CA, USA) at the Zoo & Exotic Analytes Lab (ZEAL), College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, Texas, USA. Analytes assayed were aspartate transaminase (AST), bile acids (BA), creatine kinase (CK), uric acid (UA), glucose (Glu), Ca, phosphorus (Phos), total protein (TP), albumin (Alb), globulin (Glob), potassium (K), and sodium (Na). Globulin was calculated from Alb and TP.

Statistical analysis: Analyse-it was used for data analysis (Analyse-it for Microsoft Excel [2019 Method Evaluation Edition] Analyse-it Software, Ltd.; https://analyse-it.com/; 2009). All data was evaluated for normality via histogram and Shapiro-Wilk test with $P > 0.05$ considered a Gaussian distribution. A comparison of data based on only a single independent (body measures, age, sex) and a dependent (analyte) variable was performed with the Student's *t*-test or Kruskal-Willis (KW) based on the finding of either of Gaussian or non-Gaussian data distribution, respectively. For the multiple independent variables, analysis of variance (ANOVA) or KW-ANOVA was performed with a post hoc Tukey-Kramer test in the event of Gaussian or non-Gaussian distribution, respectively. Pearson or Spearman correlations were used to assess the association of SVL and changes in UA, Phos, and Glu concentrations in the event of Gaussian or non-Gaussian data distribution, respectively. Differences were considered significant when α was <0.05. Samples collected with a result of 10% or greater (1+ or higher) for hemolysis or lipemia were analyzed for effect on analytes but were excluded from the calculation of reference intervals.

Figure 1. Restraint method for ventral coccygeal venipuncture of eastern collared lizards (*Crotaphytus collaris*) sampled in this study.

Reference intervals for the eastern collared lizard were established in accordance with the American Society for Veterinary Clinical Pathology (ASVCP) guidelines (Friedrichs *et al.*, 2012), using the parametric method. Statistical outliers, defined as less or greater than the mean by ± 3 SD were excluded from RI construction except for Ca concentrations of greater than 17 mg/dl. All CK activities greater than 10,000 units were above the limit of quantification for the analyzer and were removed for the purposes of RI construction but were converted to 10,001 for the purposes of data comparisons.

Results

Eighty-seven healthy eastern collared lizards (56 males and 31 females) had SVL, BCS, color and locale, and reproductive

Table 1. Venous blood biochemical analytes of eastern collared lizards (*Crotaphytus collaris*).

Analyte	Units	*n*	Sex	Mean ± SD	Lower 90% CI (lower limit)	Median	Upper 90% CI (upper limit)	Reference interval	Shapiro-Wilk *P* value
Aspartate† aminotransferase	U/L	72	M&F	25.8 ± 10.0	3.9–7.7	24.0	43.9–47.7	5.8–45.8	0.0637
Creatinine kinase	U/L	71	M&F	3,271.8 ± 1,689.54	0–330.0	3122.0	6,335.1–6,995.1	0.0–6665.1	0.0612
Uric Acid	mg/dL	72	M&F	7.28 ± 3.64	0.00–0.71	6.80	13.88–15.28	0.00–14.58	0.0532
Glucose	mg/dL	71	M&F	260.7 ± 37.6	177.7–192.4	261.0	329.0–343.6	185.1–336.3	0.3037
Calcium	mg/dL	68	M&F	11.28 ± 1.15	8.74–9.18	11.30	13.37–13.81	8.96–13.59	0.7768
Calcium	mg/dL	25	FG	13.28 ± 4.08	10.67	12.00	13.33		
Calcium	mg/dL	4	FO	17.25 ± 5.19	12.92	19.00	21.00		
Phosphorus	mg/dL	72	M&F	6.89 ± 1.55	3.48–4.08	6.80	9.70–10.30	3.78–10.00	0.9691
Total Protein	g/dL	72	M&F	3.98 ± 0.65	2.23–2.80	4.00	5.17–5.41	2.68–5.29	0.4350
Albumin	g/dL	73	M&F	2.78 ± 0.55	1.58–1.78	2.80	3.78–3.98	1.68–3.88	0.5720
Globulin*	g/dL	72	M&F	1.21 ± 0.34	0.47–0.59	1.10	1.84–1.96	0.53–1.90	0.0485
Potassium	mmol/L	72	M&F	5.12 ± 0.97	2.99–3.37	5.40	6.88–7.24	3.17–7.06	0.0627
Sodium	mmol/L	71	M&F	160.3 ± 7.0	144.9–147.6	161	173.0–175.7	146.3–174.3	0.741

M = male; F = female; FG = gravid female; FO = female, recent oviposition; Q = quartile; CI = confidence interval. Gray-highlighted values are calcium values for gravid females and recent oviposition females. Gray-highlighted values are estimations, not true values. * = Globulin values were determined by subtracting albumin from total protein. All analytes were normally distributed with Shapiro-Wilk $P > 0.05$.

status recorded and had biochemical analytes analyzed. Most analytes were parametrically distributed (Table 1). To the authors' knowledge, all animals remained healthy except for a single animal that died following complications associated with oviposition 3 months after sampling. Venipuncture was performed safely and without complications using manual restraint for a ventral approach to the ventral coccygeal vein.

Adult animals were larger than juveniles and males were larger than females based on weight and SVL (KW $P < 0.0001$ and $P < 0.01$ respectively) (Table 2). Although linear regression failed to find significant differences in analytes based on lizard weight (linear regression, $P = 0.5145$), as SVL increased, UA increased, and Glu decreased (Fig. 2). Similarly, UA concentrations were higher and Glu concentrations were lower in adults than those in juveniles (Fig. 3).

Uric acid concentrations differed based on lizard BCS. Lizards with a BCS of 2 had increased UA concentrations compared to that of lizards with a BCS of 3 or 5 (Fig. 4). Glucose increased as BCS increased. The mean blood Glu concentrations of BCS 5 lizards was higher than that of lizards with BCS 2 and 3, (TK post hoc $P = 0.0001$ and $P = 0.0043$ respectively) (Fig. 5). Only UA and Glu concentrations had changes associated with lizard color and locale (ANOVA, $P < 0.001$). Plasma UA concentrations of LBTX, YBNM, and YNM were increased compared to AQFOK, and LBTC lizards also had significantly higher plasma concentrations of UA than YNM lizards (Fig. 6). Although blood Glu concentrations differed based on color and locale ($P = 0.0408$), post hoc testing failed to better define this difference (data not shown).

Sex and reproductive status were associated with differing blood concentrations of Ca, Phos, K, and Alb. Calcium and Phos were higher, whereas K and Alb were lower in females than those in males (Table 3). Calcium concentrations further differed based on reproductive status (Fig. 7). Gravid and postoviposition female lizards had significantly higher blood Ca concentrations than male lizards. However, blood Ca concentrations in reproductively quiescent females did not differ from those of males (Fig. 7). Differences occurred for Phos (KW, $P = 0.0007$) based on reproductive status; however, post hoc testing failed to further define this difference, and the only reproductive categories that approached a significant difference were males and gravid females (TK, $P = 0.0682$) (data not shown). Total protein varied based on reproductive status (KW, ANOVA $P = 0.0082$) with post hoc testing supportive of a difference in female gravid and female oviposited (TK, $P = 0.0225$) and male and female gravid (TK, $P = 0.0293$) (data not shown).

No apparent effect of lipemia occurred for any analyte (KW, $P > 0.05$ for all) based on a comparison of the six lipemic samples (range, 1–2+), with all remaining samples without lipemia. The presence of apparent "icterus" (ICT), or yellow coloration within the sample ($n = 42$, all 1+), was associated with relatively decreased mean UA concentrations ($P = 0.0040$), higher mean Glu concentrations ($P = 0.0135$), higher mean Ca concentrations ($P = 0.0126$), higher mean TP concentrations ($P = 0.0082$), and higher mean

Table 2. Relevant size, age, and sex parameters for eastern collared lizards (*Crotaphytus collaris*) sampled for venous blood biochemical analytes in this study. Juvenile lizards were less than 12 months of age and adults were greater than 12 months of age. Two (aquaflame OK) measured 9.6-cm and 10.1-cm snout–vent length and one (yellow NM) male measured 9.6 cm at 11 months of age. All other juvenile males measured 7.7-cm to 9.3-cm snout–vent length.

Age	Sex	Sample size	Weight (g)	Snout–vent length (cm)	Body condition score
Juvenile	Male	19	18.8–38.4	7.7–10.1	2–5
	Female	7	21.9–28.8	8.1–8.7	2–4
Adult	Male	37	34.2–58.4	9.5–11.6	2–5
	Female	24	20.6–50.5	8.8–10.7	1–5

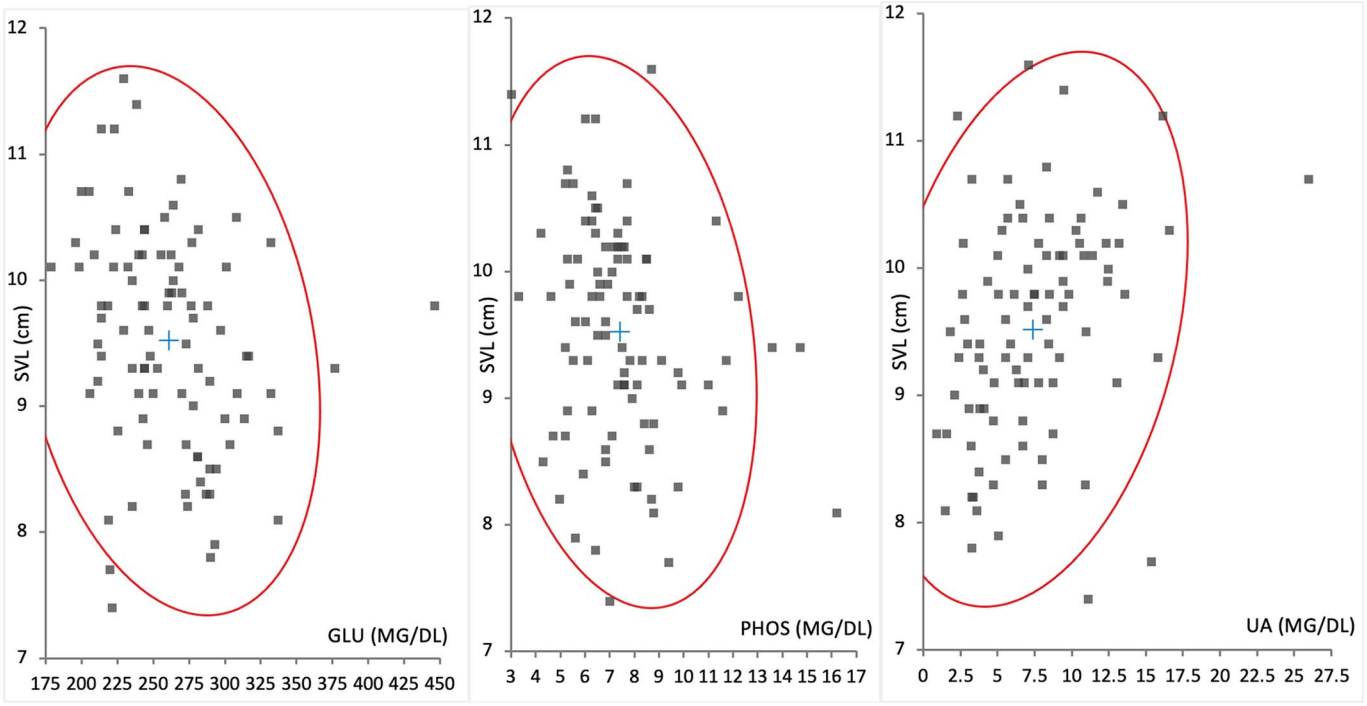

Figure 2. Multiple venous blood biochemical analytes of eastern collared lizards (*Crotaphytus collaris*) change appreciably based on lizard length. Pearson's correlations support the association of increasing glucose and phosphorous concentrations with decreased snout–vent length (SVL) in captive eastern collared lizards (a,b). For uric acid concentrations, Pearson's correlation confirmed the association of increasing SVL and increasing uric acid ($P = 0.0004$). SVL was normally distributed (SWp, 0.7348).

Alb concentrations ($P = 0.0035$) than those without yellow coloration. Animals with hemolysis had increased AST ($P = 0.0004$) and increased CK ($P \leq 0.0001$). Any result with a hemolysis or lipemia index greater than 10% was discarded from the study population. For males, with ($n = 33$) and without ($n = 23$) yellow coloration and for females with ($n = 11$) and without ($n = 20$) yellow coloration, a calculated odds ratio suggests that males are approximately twice as likely as female to have yellow coloration in the plasma, although our result failed to reach significance ($P = 0.1672$). Most samples produced a result on this analyzer that was below the limit of detection for BA. Reported BA concentrations were omitted from RI creation but were used in the statistical assessment of other categories. No apparent effect of a reported BA concentration for any analyte was calculated (KW, $P > 0.05$ for all) based on the comparison of reported BA values ($n = 13$, range = 4–30 µmol/L) to all remaining samples without a reported BA value ($n = 71$).

Discussion

Many physiological normative processes in reptiles can cause statistical differences in plasma biochemistries. Here, we have limited our discussion to those analyte values and differences found in this species compared to other reptiles, which could cause diagnostic challenges to the reptile veterinary clinician, or those that could be clinically valuable in the diagnosis of disease. Although the analyte results we present have many statistically relevant differences, we have focused our discussion to 1) Glu and UA, as values were relatively increased compared to those reported for many reptiles; 2) analyzer and rotor foibles regarding the reporting of ICT, total bilirubin, and other effects of interferents, such as hemolysis; and 3) the effect of sex for this species during reproduction, which appears to affect analytes somewhat more dramatically than other evaluated factors, possibly based on the extreme investment in vitellogenesis of the females of this lizard species.

There was a correlation in blood Glu concentrations as they increased with the higher BCS for the eastern collared lizard, and blood Glu values were relatively high for this species compared to those reported for other lizards and, indeed, most other reptiles adjudged healthy (Stahl, 2006). Blood Glu concentrations above 200 mg/dl have been touted as a rare occurrence in reptiles (Campbell, 1996). However, in this study, most collared lizards had blood Glu concentrations higher than 200 mg/dl, which are like the range of blood Glu concentrations reported for almost all western hemisphere Iguanidae, with the exception of gravid green iguana (*Iguana Iguana*) females (Table 4) (Divers *et al.*, 1996; Harr *et al.*, 2001; James *et al.*, 2006; Maria *et al.*, 2007; Dallwig *et al.*, 2011). For both eastern collared lizards and green iguanas, blood Glu concentrations were relatively increased in juveniles compared to adults. In bearded dragons, an arid omnivore, Glu was higher in males in the breeding season than that in males outside the breeding season (Howard and Jaensch, 2021). As evidenced by the many egg-bearing females we sampled, the lizards in our

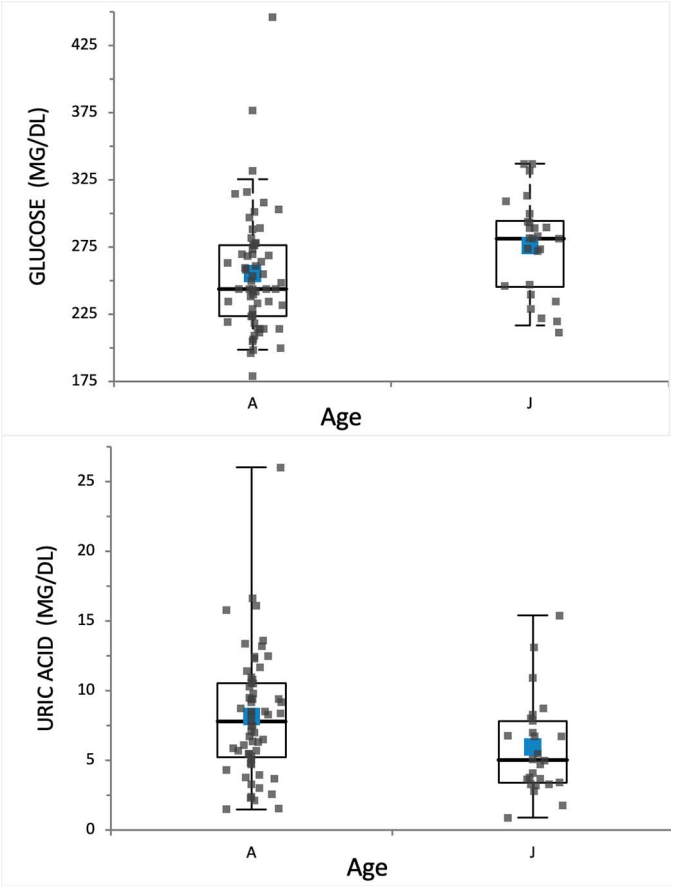

Figure 3. Glucose concentrations were lower and uric acid concentrations were higher in adult (A) eastern collared lizards (*Crotaphytus collaris*) than those in juveniles (J) (Kruskal-Wallis $P = 0.0057$ and $P = 0.0131$ respectively). In this box-and-whisker plot, the median is shown as a thick black line, the mean is shown as a filled square, the box represents the 5%–95% quantiles of the median, and the whiskers represent the full range of the data, with each data point represented as a dot.

study were captured and sampled during their breeding season. Other possible causes of this relatively increased blood Glu for the iguanids compared with that reported in other species could include a lack of fasting before sampling, the stress of captivity, the stress of capture or handling for venipuncture, or a naturally relatively high metabolic rate associated with their preferred high temperatures and these active species' proclivity toward aggression, territoriality, and rapid movement. Additional study of this and other closely related species is recommended regarding this normative "hyperglycemic" phenomenon of Iguanids.

Adult lizards with a lower BCS and some color morphs vs AQFOK had relatively increased UA concentrations. Uric acid values for these apparently healthy eastern collared lizards ranged well above those of other western hemisphere iguanids. In the Dickerson's collared lizard, multiple factors and color traits play a role in physical and physiological performance (Plasman *et al.*, 2015). However, our comparison of blood biochemistry values based on color traits in the eastern collared lizard revealed only variations of blood UA concentrations. The arid habitat this species occupies and its carnivorous and insectivorous nature may, in part, explain these relatively high UA values. In addition, these lizards were fed 24 h before sampling, and in inland bearded dragons, mean plasma UA concentration for lizards fed a cricket increased at 24 h after meal ingestion (Parkinson and Mans, 2020). Obversely, fasting tegus presented higher UA levels than fed tegus (Gavira *et al.*, 2018). Arid environmental adaptation may also play a role in the relatively high UA values we report from this species, as the Gila monster (*Heloderma suspectum*) and savannah monitor (*Varanus exanthematicus*), carnivorous reptiles from similarly arid locales, and the veiled chameleon *Chamaeleo calyptratus*, a relatively arid insectivore compared to most *Chamaeleo* spp., have similar reported plasma UA concentrations (Cooper-Bailey *et al.*, 2011; Sladky, 2022). Further studies are needed to determine the effects, if any, of feeding schedule upon biochemistry analytes in lizards; the biochemistry RI presented for eastern collared lizards may not describe values for animals that are immediately postprandial or that have been fasted or experienced prolonged anorexia.

As this species is not known to produce biliverdin reductase, we suggest that the increased yellow color of plasma resulting in the classification of ICT by the VS2 analyzer is likely related to circulating carotenoids, which could increase in females to support vitellogenesis. In green iguanas, oxygenated carotenoids (xanthophylls) including lutein, zeaxanthin, and canthaxanthin occur in the plasma and can impart yellow, yellow-to-red, and reddish-orange colors to the plasma, respectively. We suspect that a similar circumstance occurs for eastern collared lizard plasma, with carotenoids being obtained from the insectivorous diet (Raila *et al.*, 2002). As males were more often labeled as having ICT plasma based on the odds ratio, perhaps the necessary deposition of carotenoids into the yolk of the egg persistently drains females of beta-carotenes and allows males of this species to retain a more colorful outward appearance and more colorful plasma (Dierenfeld *et al.*, 2002; San-Jose *et al.*, 2012). Interestingly, ICT plasma was associated with many factors likely related to increased health or fitness and includes decreased UA concentrations and increased Glu, Ca, TP, and Alb concentrations. Practitioners should be aware that an ICT sample in an eastern collared lizard, and possibly other iguanids, could be a sign of better health rather than an indicator of failing health status.

The association of hemolysis with increased AST and CK activities and increased UA concentrations was somewhat expected. The relatively common finding of mild hemolysis was unexpected but may have resulted from our restraint and collection method to include manual restraint of the tail and the use of relatively small gauge needles by a relatively large-handed person (BJL). In green iguanas, blood samples with marked hemolysis had increased AST activity but also increased concentrations of Phos, K, and TP, which were not evident based on our analysis (Benson *et al.*, 1999). Similarly, additional bands of protein were noted in hemolyzed samples from green iguanas analyzed via protein electrophoresis

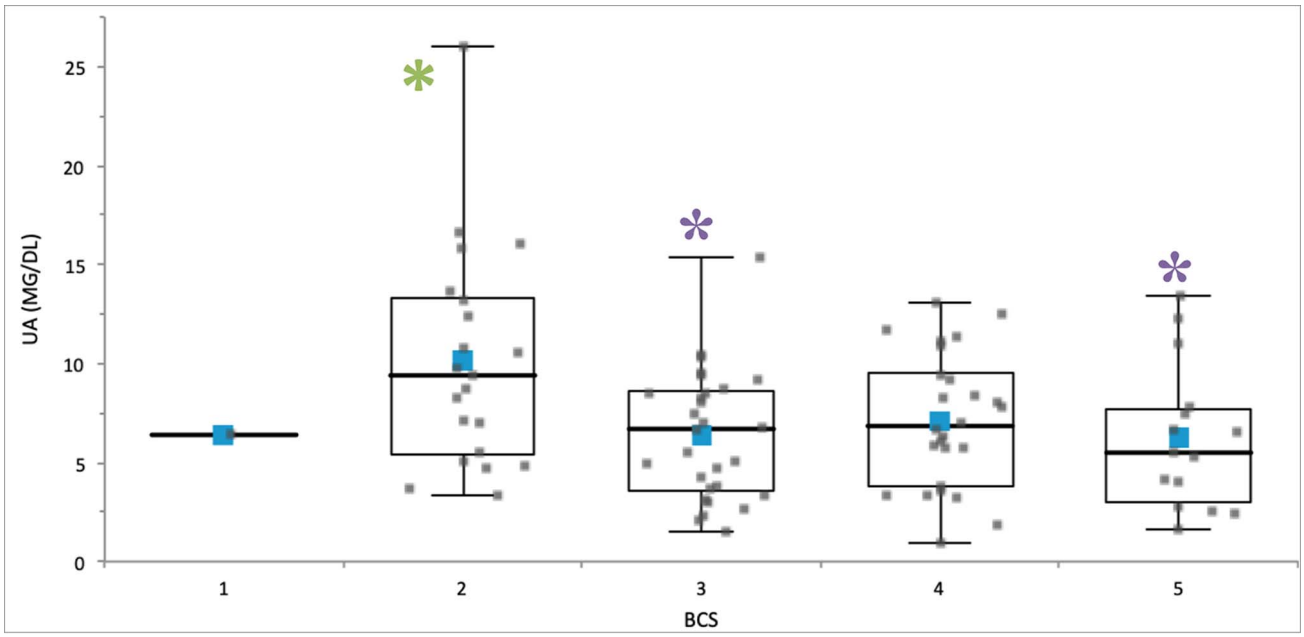

Figure 4. Uric acid concentrations were associated with changes in body condition score (BCS) of eastern collared lizards (*Crotaphytus collaris*) (Kruskal-Wallis ANOVA $P = 0.0156$) and post hoc testing (Tukey-Kramer multiple comparisons of the means) supported that uric acid of lizards with a BCS of 2 was increased compared to that of lizards with BCSs of 3 and 5, respectively (post hoc Tukey-Kramer $P = 0.0208$ and $P = 0.0326$). In this box-and-whisker plot, the median is shown as a thick black line, the mean is shown as a filled square, the box represents the 5%–95% quantiles of the median, and the whiskers represent the full range of the data, with each data point represented as a dot.

(Giménez *et al.*, 2010). However, the VetScan VS2 manual states that "AST samples may be artificially high due to in-vitro hemolysis," without specifying species [VS2 Manual]. Increased CK activities could relate to increased animal activity or difficulty of the venipuncture for the collection for these samples, as has been hypothesized for green iguanas (Grant *et al.*, 2009). The *Crotaphytus* genus does not display caudal autotomy; therefore, sedation is not a requirement for

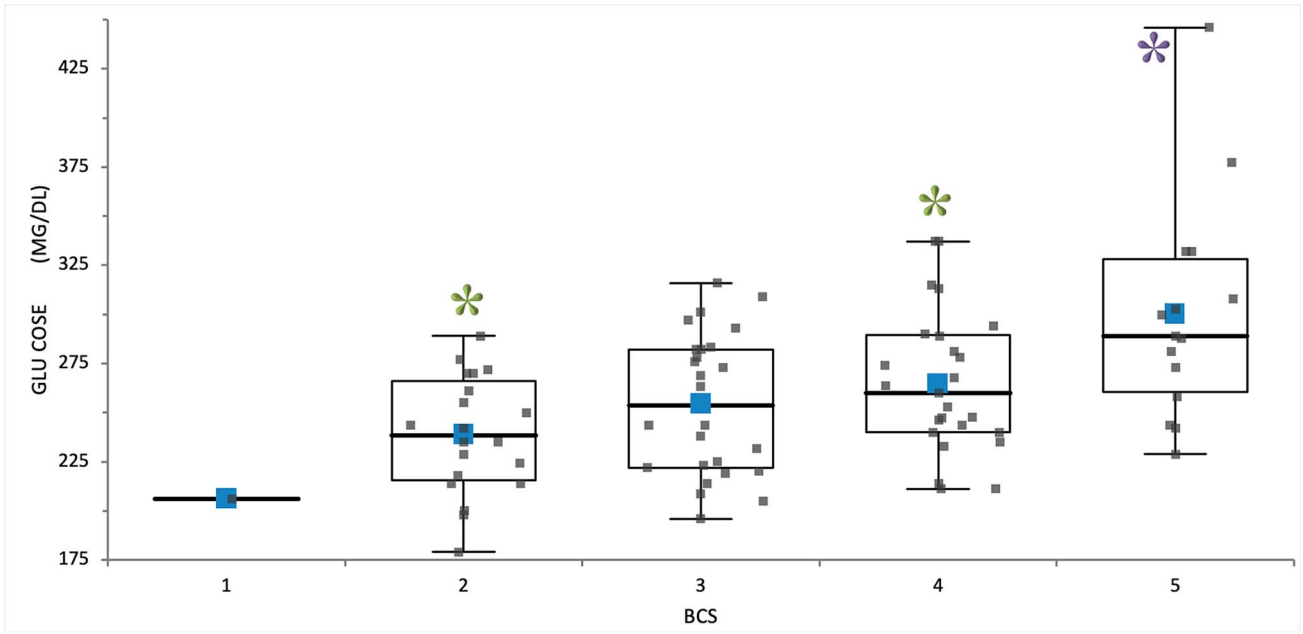

Figure 5. As lizard body condition score (BCS) increased, blood glucose also increased in eastern collared lizards (*Crotaphytus collaris*) (Kruskal-Wallis $P = 0.0002$). Post hoc testing supported that the mean glucose of lizards with a BCS of 5 was higher than that of lizards with a BCS of 2 or 3 (post hoc Tukey-Kramer $P = 0.0001$ and $P = 0.0043$, respectively). In this box-and-whisker plot, the median is shown as a thick black line, the mean is shown as a filled square, the box represents the 5%–95% quantiles of the median, and whiskers extend to the full range of the data, with each data point represented as a dot.

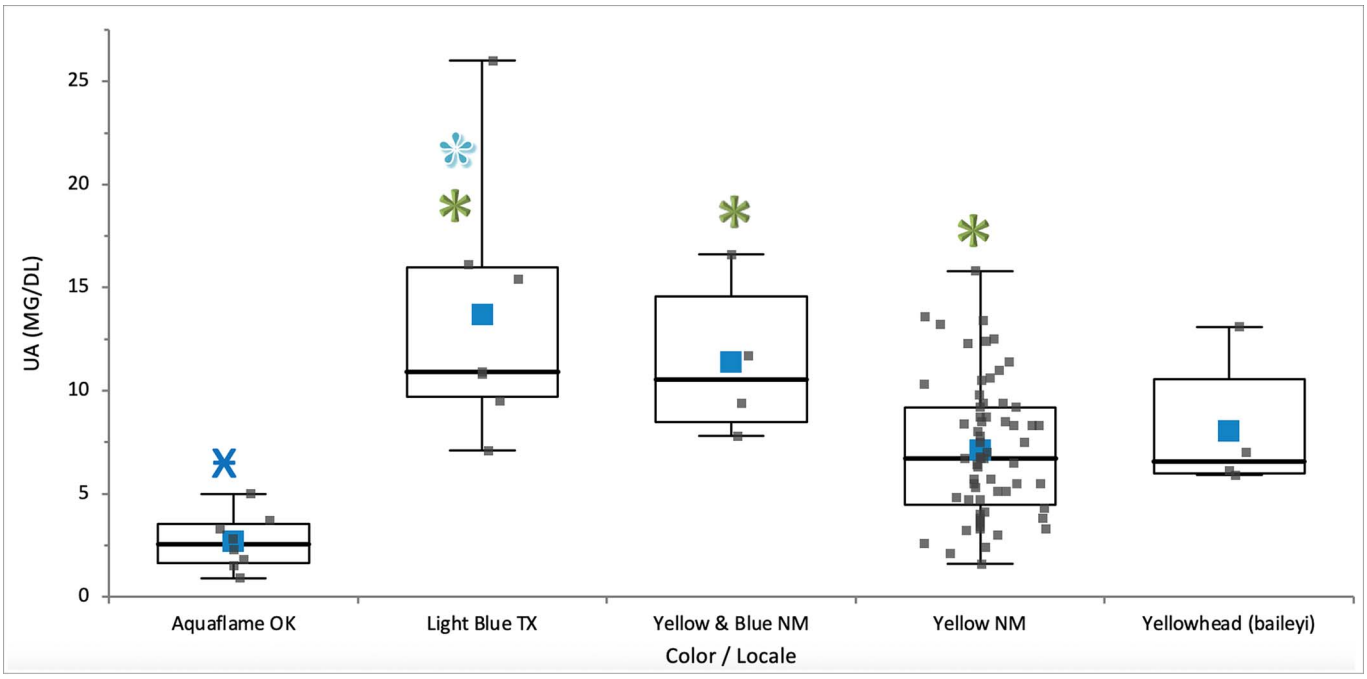

Figure 6. Differing uric acid concentrations of eastern collared lizards (*Crotaphytus collaris*) based on color and/or original locale. Plasma uric acid concentrations of AQFOK were significantly decreased compared to those lizards from Texas and New Mexico (post hoc Tukey-Kramer $P < 0.0001$, 0.0092, 0.0010, respectively). LBTX lizards also had significantly higher blood concentrations of uric acids than YNM lizards (TK $P < 0.001$). The median is represented by a thick black line, the mean is a filled square, the box represents the 5%–95% quantiles of the median, and the whiskers extend through the data range, with each data point represented as a dot.

ventral coccygeal venipuncture of this species but might have reduced the values for the analytes based on a lesser need for restraint for sampling (Fitch, 2003). Finally, increased values for AST and CK likely relate to increased concentrations of these analytes inside the red blood cells of eastern collared lizards. However, further studies of hemolyzed samples would be necessary to support this hypothesis.

Calcium, Phos, and K were higher and Alb was lower in females than those in males. Relatively increased concentrations of Ca and Phos in females were expected, as many of these female lizards were in the process of vitellogenesis. Similar findings have been evident in biochemical analytes of other iguanids; our gravid female eastern collared lizards had similar Ca concentrations as those of gravid female iguanas (Harr *et al.*, 2001). The finding of lower TP and Alb in female eastern collared lizards was unexpected and is a contrast to the findings in one study of green iguana biochemistry analytes using the VS2 analyzer (Grant *et al.*, 2009). However, investment in reproduction is large in this species, and even first-year females are known to double clutch and produce five eggs per clutch, whereas second-year and older females produce up to nine eggs and may treble clutch (Ballinger and Hipp, 1985). Although this characteristic makes these lizards desirable for the pet trade, this extreme reproductive investment likely results in relatively reduced concentrations of plasma Alb. Furthermore, testosterone supplementation of male lacertid Algerian psammodromus (*Psammodromus algirus*) increased their blood protein concentrations (Puerta *et al.*, 1996). Although first-year male eastern collared lizards have relatively increased testosterone

Table 3. Blood biochemical analytes that differ based on sex for eastern collared lizards (*Crotaphytus collaris*). Non-Gaussian analyte distribution was limited to potassium (†) for which the statistic used was the Kruskal-Wallis test. For all other analytes, Student's *t*-test was used.

Sex	Analyte (units)	Minimum	1st Quartile	Median	3rd Quartile	Maximum	*P* value
Female	Calcium (mg/dl)	9.2	10.70	12.10	19.33	21.0	<0.0001
Male		8.3	10.32	11.30	11.98	13.5	
Female	Phosphorus (mg/dl)	4.7	7.13	8.00	9.05	16.2	0.0003
Male		3.0	5.64	6.55	7.66	12.2	
Female	Potassium† (mmol/L)	1.4	5.12	5.60	6.00	6.6	0.0168
Male		2.9	4.20	5.10	5.70	7.5	
Female	Albumin (g/dl)	1.2	2.20	2.40	2.98	4.1	0.0405
Male		1.7	2.44	2.85	3.20	4.4	

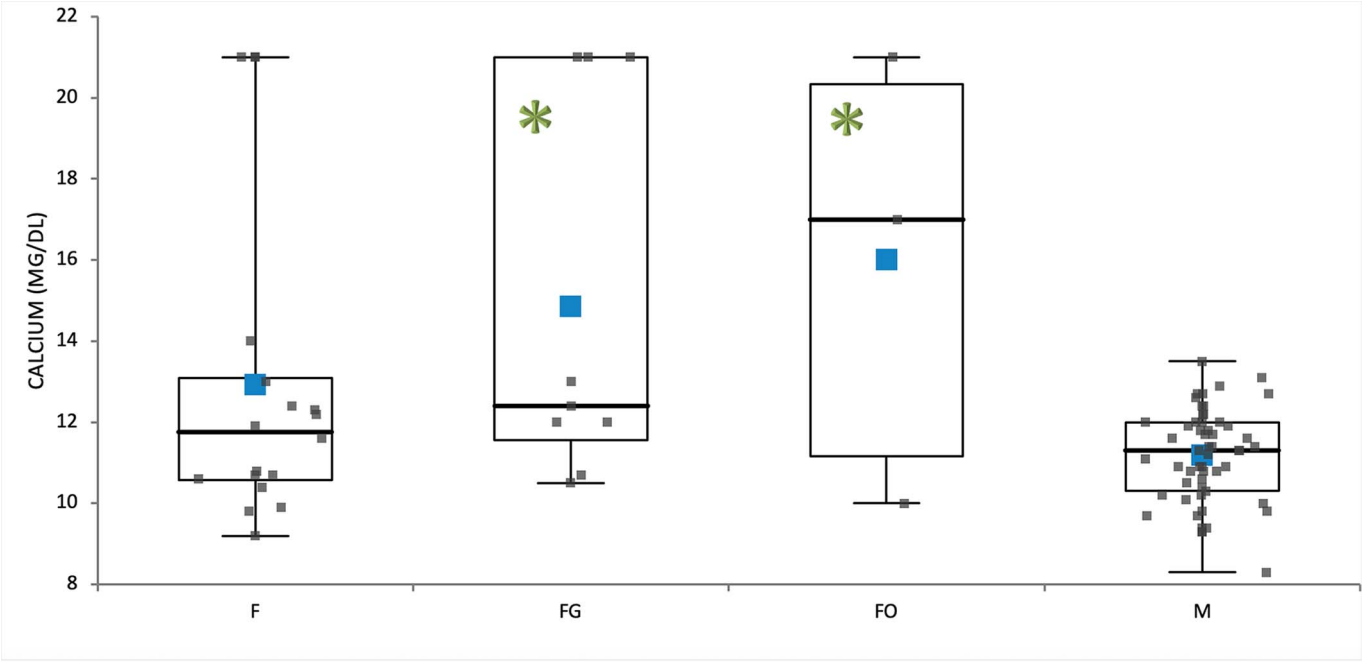

Figure 7. Blood calcium concentrations of eastern Collared Lizards (*Crotaphytus collaris*) vary based on sex and reproductive status (Kruskal-Wallis ANOVA P =0.0459). Gravid (FG) and postoviposition (FO) female lizards have significantly higher blood calcium concentrations than male (M) lizards (post hoc Tukey-Kramer P = 0.0013 and P = 0.0149, respectively). However, reproductively quiescent females (F) do not have blood calcium concentrations that significantly differ from those of males. The median of the data is shown as a thick black line, the mean is shown as a filled square, the box represents 5%–95% quantiles of the median, and the whiskers extend to the full range of the data, with each data point represented as a dot.

concentrations compared to older males (Baird and Hews, 2007), we did not find evidence of any effect on TP based on age in male collared lizards. Potassium differences between male and female collared lizards were clinically small; however, the findings of an approximately three-fold increase of K in eggs compared with that in neonates (0.12 ± 0.017 in neonates, 0.40 ± 0.01 mg in eggs) for metallic cool-skinks (*Niveoscincus metallicus*) does suggest that egg creation in lizards is a K-dependent process (Ramírez-Pinilla, 2006). Overall, we suggest that all of the differences observed for males versus females were likely related to the body-resource-intensive reproductive process in the females of this species, which was in full bloom at the time of sampling for these adult lizards.

Limitations of this study include rotor limits of detection and known analyzer inaccuracy for some analytes. The VetScan VS2 Abaxis avian/reptile profile plus rotor was unable to provide values below a BA of 35 µmol/l. In retrospect, the use of the mammalian liver profile (MLP) rotor, which has a lower limit of detection for BA, for the determination of a BA RI for this species would be recommended. However, the small size of these lizards limited our ability to obtain an adequate sample volume necessary for the two rotors (Clarizio *et al.*, 2022). Methods used in a previous study on BA may be needed to properly evaluate BA levels in the *Crotaphytus* genus (Hagey *et al.*, 2010). Analyzer limitations also continue to affect the veterinarian's ability to evaluate Alb, Glob, and TP of exotic animals. Abaxis Vetscan VS2 biochemistry analyzers are not reliably accurate in the determination of Alb and Glob concentrations for exotic animals (Greenacre *et al.*, 2008). Although protein electrophoresis is considered the most accurate tool for the determination of TP, the lack of quantitative values provided, a current lack of a singular assay method, and expense make this assay less accessible and challenging to interpret when used for reptiles. In retrospect, a comparison of total solids as determined by a refractometer and total solids as determined by the VS2 could have provided valuable information to this study. However, we were limited by sample size and the relatively increased values for UA and Glu for this species, and the presence of lipemia in some samples might have interfered with such efforts.

Conclusions

This study established biochemistry RIs for plasma concentrations for a reptile plasma biochemical panel of analytes for apparently healthy eastern collared lizards. These biochemical analyte RIs can be used to evaluate the health of eastern collared lizards and possibly other closely related lizard species. Values for Glu and UA ranged higher than those previously reported for other western hemisphere iguanids, but they were not outside those reported for other high activity and/or arid adapted carnivorous lizards and could also reflect recent meal ingestion. The RIs provided should be considered analyzer specific, as constant and proportional bias variation occured in a comparison of different analyzers (McCain *et al.*, 2010). The RIs created here for eastern collared lizards may not represent the entire genus of collared lizards, and

Table 4. Comparative biochemistry ranges for the eastern collared lizard, *Crotaphytus collaris*; compared to analyte ranges of the green iguana, *Iguana iguana* (a: Divers *et al.*, 1996, b: Harr *et al.*, 2001); the plumed basilisk, *Basiliscus plumifrons* (c: Dallwig *et al.*, 2011); the Allen Cays rock iguana, *Cyclura cychlura inornata* (d: James *et al.*, 2006); and the Ricord's iguana, *Cyclura ricordii* (e: Maria *et al.*, 2007). –, Data not provided. Biochemistry analytes include aspartate aminotransferase (AST), creatine kinase (CK), uric acid (UA), glucose (Glu), calcium (Ca), phosphorus (Phos), total protein (TP), albumin (Alb), globulin (Glob), potassium (K), and sodium (Na).

Species/source	AST (U/L)	CK (U/L)	UA (mg/dl)	Glu (mg/dl)	Ca (mg/dl)	Phos (mg/dl)	TP (g/dl)	Alb (g/dl)	Glob (g/dl)	K (mmol/L)	Na (mmol/L)
Crotaphytus collaris	5.45.8	0–6,665.1	0.00–14.58	185.1–336.3	8.96–13.59	3.78–10.00	2.68–5.29	1.68–3.88	0.53–1.90	3.17–7.06	146.3–174.3
Iguana iguana[a]	5–52	–	0.79–1.58	169–288	39.6–63.1	27.0–54.1	5–7.8	2.1–2.8	2.5–4.3	–	–
Iguana iguana[b] (male)	19–65	–	1.5–5.8	70–244	8.6–14.1	3.2–7.6	4.4–6.5	1.3–3.0	2.5–4.4	2.8–6.1	152–162
Iguana iguana[b] (gravid female)	12–29	–	2.2–4.9	126–153	22.5–47.5	8.4–17.5	7.0–8.2	1.5–2.5	4.9–6.7	3.2–5.0	161–164
Iguana iguana[b] (female)	7.0–102	–	0.9–6.7	105–258	10.8–14.0	2.8–9.3	4.9–7.6	1.5–3.0	2.8–5.2	2.0–5.8	156–172
Iguana iguana[b] (juvenile)	13.0–72	–	0.7–5.7	131–335	12.1–23.2	4.3–9.0	4.2–6.1	2.0–2.8	2.2–3.0	–	–
Basiliscus plumifrons[c]	19.5–115	2,497–8,893	0.6–2.9	108–279	8.3–12.4	4.1–9.3	3.1–6.6	1.3–2.6	1.6–4.7	2.3–7.9	142–167
Cyclura cychlura inornata[d]	13.09–45.85	0–4,914	0–3.77	149.86–228.58	3.83–22.23	3.01–7.61	3.92–5.68	1.65–2.41	2.27–3.45	2.60–4.72	160.93–172.91
Cyclura ricordii[e]	12.3–70.9	1.08–6,548.84	0.49–3.40	169.2–270.0	46.8–64.8	25.2–39.6	6.14–8.18	1.85–2.45	4.2–6.0	2.32–4.92	148.5–177.5

further research is needed to establish that other species in the genus have similar biochemical RIs (McGuire, 1996).

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