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Biochemical reference intervals for captive bred *Crocodylus moreletii* and *Crocodylus acutus* in the Alcuzahue Lagoon, Colima, Mexico

[Intervalos de referência bioquímica para Crocodylus moreletii *e* Crocodylus acutus *criados em cativeiro na Lagoa Alcuzahue, Colima México]*

R.O.F. *Prado*, D.M.I. *Carrillo*, R.J.A. *Hernández*, R.J.A. *Ruíz*, C.A.C. *García**

Facultad de Medicina Veterinaria y Zootecnia, Universidad de Colima, Colima, México

ABSTRACT

Crocodylus moreletii and *Crocodylus acutus* are two endangered crocodile species endemic to Mexico. In this descriptive cross-sectional study, a total of 58 crocodiles (30 females and 28 males) were examined to determine and evaluate 24 blood biochemical indicators associated with energy, lipid, protein, mineral and enzymatic metabolic processes. Most of the serum biochemistry parameters were similar among sexes by species. However, male crocodiles showed higher triacylglycerol concentration and alkaline phosphatase activity, and lower globulin concentration than females. There were some significant differences between species. Total bilirubin, triacylglycerol, carbon dioxide, and hydrogen carbonate were higher in *Crocodylus moreletii*, and urea nitrogen, creatinine, alanine aminotransferase, and aspartate aminotransferase were higher in *Crocodylus acutus*. These reference values are very important for the protection of crocodiles. The calculated confidence intervals could be used to detect alert situations when at least 5% of the sampled crocodiles would fall outside of the calculated reference interval for a given parameter.

Keywords: blood chemistry, metabolic profile, *Crocodylus moreletii*, *Crocodylus acutus*

RESUMO

Crocodylus moreletii *e* Crocodylus acutus *são duas espécies de crocodilos ameaçadas de extinção endêmicas do México. Neste estudo transversal descritivo, um total de 58 crocodilos (30 fêmeas e 28 machos) foi examinado para determinar e avaliar 24 indicadores da bioquímica sanguínea associados a processos metabólicos energéticos, lipídicos, proteicos, minerais e enzimáticos. A maioria dos parâmetros bioquímicos séricos foram semelhantes entre os sexos da espécie. No entanto, os crocodilos machos apresentaram maior concentração de triacilgliceróis e atividade de fosfatase alcalina do que as fêmeas. Com uma concentração de globulina mais baixa do que as fêmeas, houve algumas diferenças significativas entre as espécies. A bilirrubina total, os triacilgliceróis, o dióxido de carbono e o bicarbonato de sódio foram maiores em* Crocodylus moreletii, *e o nitrogênio ureico, a creatinina, a alanina aminotransferase e o aspartato aminotransferase foram maiores em* Crocodylus acutus. *Esses valores de referência são muito importantes para a proteção dos crocodilos. Os intervalos de confiança calculados podem ser usados para detectar situações de alerta quando pelo menos 5% dos crocodilos amostrados estiverem fora do intervalo de referência calculado para um determinado parâmetro.*

Palavras-chave: bioquímica sanguínea, perfil metabólico, Crocodylus moreletii, Crocodylus acutus

INTRODUCTION

The natural wealth of planet earth is estimated at 1,700,000 species (Zamorano, 2009). Mexico is one of the five countries with the greatest biological diversity (Cántaro and Cántaro, 2020). Worldwide, it ranks 2nd in terms of diversity in reptiles (n = 804 spp.), with 173 endemic species (Sánchez, 2011). In this group, crocodiles are aquatic reptiles adapted to water-land interfaces such as swamps, ponds, rivers, and lagoons (Padilla and Weber, 2016). In the Mexican territory the crocodile population was greatly

*Corresponding autor: cesargarciacasillas@hotmail.com
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reduced in many areas due to unregulated skin hunting, which occurred mainly in the 1970s (Cedillo *et al.*, 2019). *Crocodylus moreletii* and *Crocodylus acutus* are protected by law and are included in the Official Mexican Directive (NOM-059-ECOL-2001), under the list of species that are subject to special protection. They are also listed in appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Convention…, 2021) and in the vulnerable category by the International Union for Conservation of Nature (Elbers, 2011). Therefore, Mexico established captive breeding programs in the Wildlife Management Unit (UMA-La Colorada), in the Alcuzahue Lagoon, Colima. Biochemical profile is a relatively non-invasive method, its blood parameters such as energy, protein, minerals, and enzymes could provide information on internal organs, nutritional status, and metabolic state (Puppel and Kuczyńska, 2016). Given their rich diversity, they are a strategic tool in animal resource conservation programs (Brakes *et al.*, 2019). However, to date, there is still not a comprehensive and complete reference range on biochemistry parameters for *Crocodylus moreletii* and *Crocodylus acutus*. Therefore, the study was planned to generate the reference values in the serum biochemical profile of captive bred *Crocodylus moreletii* and *Crocodylus acutus* in the Alcuzahue Lagoon, Colima Mexico.

MATERIALS AND METHODS

This work was conducted as part of a population health assessment approved and supported by the Network Advances in Agricultural Research in Mexico. All the handling and sampling were performed in compliance with standard vertebrate protocols and veterinary practices, and in accordance with Bioethics and Animal Welfare Commission of the Faculty of Veterinary Medicine and Zootechnics of the University of Colima. Evaluation act: No. 1, February 3, 2021.

Fifty-eight crocodiles: 29 *Crocodylus moreletii* and 29 *Crocodylus acutus* (15 females and 14 males per species), were captured in the Alcuzahue Lagoon of the Wildlife Management Unit (UMA-La Colorada), located on 18km east of Tecomán (Environmental…, 2021), 47m snm altitude (Figure 1), with a warm sub-humid climate (Köppen Cfb) (Peel *et al.*, 2007), temperature between 12.1°C in Jan and 37.1°C in May with rainfall of 484mm/year (Sedesol, 2021). Capture was achieved using aluminum poles with a stainless-steel cable, none of the crocodiles were sedated to avoid the interference of anesthetic drugs on the blood parameters.

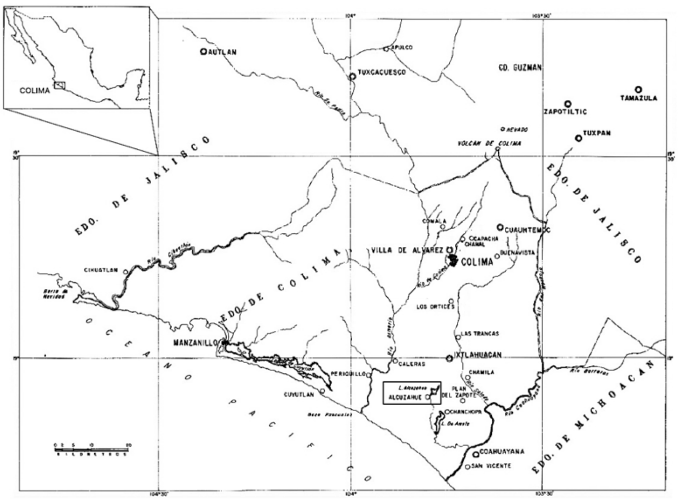

Figure 1. Location of Alcuzahue Lagoon of the Wildlife Management Unit; UMA-La Colorada., Colima, Mexico.

The crocodiles were examined for clinical signs of trauma and illness, and the gender was determined by digital palpation of genital organs in the cloacae (Ziegler and Olbort, 2007). The average age was 3 years, weight 70.60±17.50kg (males), 60.10±7.50kg (females), and length

250.20±30.15cm for both sexes. Crocodiles were fed once a week, primarily on locally available fresh fish, chicken, and bovine viscera (lung and heart).

Immediately after capture, blood was taken from the post-occipital venous sinus located at the dorsal midline and caudal to the base of the head (Ragaza *et al.*, 2021) using sterile 10mL syringes with 20 g needles (Figure 2).

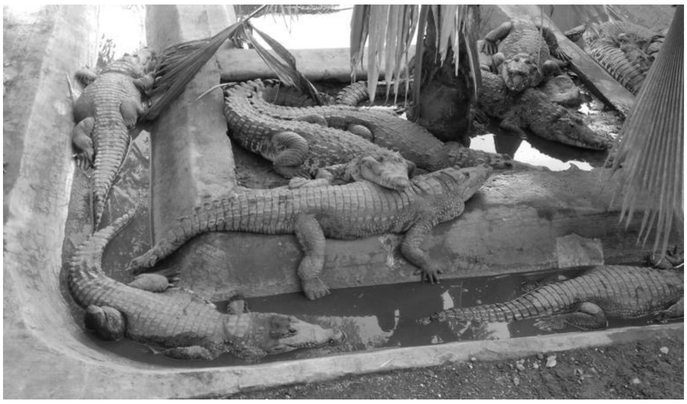

Figure 2. Post-sampling isolated crocodiles. Alcuzahue Lagoon of the Wildlife Management Unit; UMA-La Colorada., Colima, Mexico.

Blood was aliquoted in 8.5mL vacuum tubes with clot activator and serum separator gel (BD Vacutainer 367988; Becton-Dickinson Co., Franklin Lakes, United States). Prior to blood collection, all individuals were fasted for 24h to reduce the possibility of obtaining highly lipemic samples. To obtain serum, the blood samples were centrifuged directly at the UMA-La Colorada, at 1 500 x *g* for 10min using a portable centrifuge (Porta-Spin C828; UNICO., Dayton, United States). Subsequently, the serum samples were separated using 1.5mL tubes with lid (Tubes Safe-Lock 3810X; Eppendorf., Madrid, Spain) and transported at 4°C in a portable refrigerator (Thermoelectric Cooler Car/Home M5644-710; Coleman Company., Kansas, United States) to the clinical laboratories at the Autonomous Metropolitan University and the University of Colima where they were frozen at -20°C until analysis.

The concentration of each analyte was determined with an UV-Vis double beam spectrophotometer (Biochemistry Analyzer ES-218; KONTROLab., Guidonia, Italy). The biochemical analytes, the analytical method for each parameter, the units in which the results were expressed, and the corresponding commercial reagents, are described in Table 1.

The precision and reliability of the techniques was controlled using lyophilized control serum (SPINTROL NORMAL 1002100; Spinreact., Girona, Spain) and (Assayed Multi-Sera AL 1027; Randox Laboratories., Northern Ireland, United Kingdom). Duplicate tests were performed for every specimen in each parameter. Hemolysis of serum was recorded on a qualitative scale of 0 (none) to 3 (dark). Samples showing hemolysis scores of 2 and above constituted less than 2% of all samples and did not introduce a significant bias in any of the tested models; thus, the influence of serum hemolysis was ignored.

We processed the data using software SPSS (2013). The statistical procedure used to calculate 95% confidence intervals for the biochemical analytes follow the recommendation of the International Federation of Clinical Chemistry (Solberg, 1987). All variables were tested for normality distribution (Shapiro-Wilk test) and homogeneity of variances (Levene test). The results were compared among sexes within each species and within species. Significant difference between means was determined using an independent sample t-test model (SPSS…, 2013). The linear relationships between the biochemical analytes were identified using a Pearson Correlation Coefficient matrix. Results were considered significant at ($P<0.05$).

Table 1. Biochemical analytes, units, analytical methods, and corresponding commercial reagents

Analyte*	Unit	Method	Reagent
Energy-lipid profile			
Glucose (GLU)	mM	Trinder. GOD-POD[1]	1001190[a]
Total cholesterol (COL-T)	mM	CHOD-POD. Liquid[2]	41020[a]
Total bilirubin (TB)	μM	DMSO. Colorimetric[3]	1001044[a]
Triacylglycerol (TAG)	mM	GPO-POD. Liquid[4]	41032[a]
β-hydroxybutyrate (β-HBA)	mM	Enzymatic[5]	RB1007[b]
Protein profile			
Blood urea nitrogen (BUN)	mM	Urease-GLDH. Kinetic UV[6]	1001333[a]
Albumin (ALB)	g/L	Bromcresol green. Colorimetric	1001020[a]
Globulin (GLOB)	g/L	(PROT-T) – (ALB)	Difference
Creatinine (Cre)	μM	Jaffé. Colorimetric-kinetic[7]	1001111[a]
Total protein (PROT-T)	g/L	Biuret. Colorimetric[8]	1001291[a]
Mineral-electrolyte profile			
Calcium ion (Ca^{2+})	mM	Arsenazo III. Colorimetric	CA2391[b]
Inorganic phosphate (P_i)	mM	Phosphomolybdate. UV[9]	1001155[a]
Sodium ion (Na^+)	mM	Enzymatic. Colorimetric[10]	1001385[a]
Potassium ion (K^+)	mM	UV Test[11]	1001395[a]
Magnesium ion (Mg^{2+})	mM	Xylidyl Blue. Colorimetric[12]	1001286[a]
Chloride ion (Cl^-)	mM	Thiocyanate-Hg. Colorimetric[13]	1001360[a]
Carbon dioxide (CO_2)	mM	Enzymatic[14]	CD127[b]
Hydrogen carbonate (HCO_3^-)	mM	Enzymatic by CO_2 total and gas dissolved	99852[c]
Anion gap (A-gap)	mM	$[(Na^+ + K^+) – (Cl^- + HCO_3^-)]$	Difference
Enzyme profile			
Alanine aminotransferase (ALT)	U/L	NADH. Kinetic UV. IFCC rec. Liquid[15]	41274[a]
Alkaline phosphatase (ALP)	U/L	p-Nitrophenylphosphate. kinetic. Liquid[16]	41233[a]
Aspartate aminotransferase (AST)	U/L	NADH. Kinetic UV. IFCC rec. Liquid[17]	41264[a]
Creatine kinase (CK)	U/L	NAC. Kinetic UV. Liquid[18]	41250[a]
γ-glutamyl transferase (γ-GT)	U/L	Carboxy substrate. Kinetic. Liquid[19]	41288[a]

*Official abbreviation of the International Union of Pure and Applied Chemistry; [1]Glucose oxidase-peroxidase; [2]Cholesterol oxidase-peroxidase; [3]Solubilization with dimethylsulfoxide; [4]Glycerol-3-oxidasa-peroxidase; [5]β-hydroxybutyrate dehydrogenase; [6]Urease-glutamate dehydrogenase; [7]Sodium picrate; [8]Copper salts in an alkaline medium; [9]Ammonium molybdate-phosphomolybdate; [10]Galactosidase-o-nitrophenyl-d-galactopyranose; [11]Pyruvate kinase-phosphoenolpyruvate; [12]Magon sulfonate in alkaline solution; [13]Mercuric thiocyanate-red ferric thiocyanate; [14]Phosphoenolpyruvate-phosphoenolpyruvate carboxylase-malate dehydrogenase; [15]α-ketoglutarate-glutamate-piruvate-lactate-NADH; [16]Pnitrophenyl phosphate-p-nitrophenol-phosphate; [17]Aspartate-α-ketoglutarate-glutamate-oxalacetate-malate-NADH; [18]Creatine phosphate-ADP-creatine-ATP-D-glucose-ADP-glucose-6-phosphate-NADP-6-phosphogluconate-NADPH; [19]γ-L-glutamyl-3-carboxy-4-nitroanilide-glycylglycine-γ-L-glutamyl-glycylglycine-2-nitro-5-aminobenzoic acid; [a]Spinreact., Girona, Spain; [b]Randox Laboratories., Northern Ireland, United Kingdom; [c]Biolabo Laboratory., Grandcamp-Maisy, France.

RESULTS

The descriptive statistic for glucose (GLU), total cholesterol (COL-T), total bilirubin (TB), triacylglycerol (TAG), β-hydroxybutyrate (β-HBA), blood urea nitrogen (BUN), albumin (ALB), globulin (GLOB), creatinine (Cre), total protein (PROT-T), calcium ion (Ca^{2+}), inorganic phosphate (P_i), sodium ion (Na^+), potassium ion (K^+), magnesium ion (Mg^{2+}), chloride ion (Cl^-), carbon dioxide (CO_2), hydrogen carbonate (HCO_3^-), anion gap (A-gap), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), and γ-glutamyl transpeptidase (γ-GT), determined from 58 blood serums of 29 *Crocodylus moreletii* and 29 *Crocodylus acutus*, and its respective international range values are shown in Table 2.

Table 2. Serum biochemistry parameters of crocodiles sampled in the Alcuzahue Lagoon of the Wildlife Management Unit; UMA-La Colorada., Colima, Mexico (n = 29 *Crocodylus moreletii* and 29 *Crocodylus acutus*, respectively)

	Crocodylus moreletii		*Crocodylus acutus*		
	x ± SD	CI	x ± SD	CI	Reference
Energy-lipid profile					
Glucose (mM)	3.90±0.47	3.72-4.08	4.02±0.45	3.85-4.19	3.24±0.30[¥]
Total cholesterol (mM)	6.14±0.77	5.84-6.43	5.95±0.71	5.68-6.22	6.21±0.94[†]
Total bilirubin (μM)	1.24±0.08	1.20-1.27	1.19±0.06	1.16-1.21	1.20±0.47[†]
Triacylglycerol (mM)	8.92±0.40	8.76-9.07	7.59±0.35	7.45-7.72	9.02±1.07[†]
β-hydroxybutyrate (mM)	0.85±0.14	0.79-0.91	0.79±0.19	0.72-0.86	
Protein profile					
Blood urea nitrogen (mM)	0.47±0.03	0.45-0.48	0.52±0.01	0.52-0.53	0.44±0.07[†]
Albumin (g/L)	26.25±0.32	26.12-26.37	26.31±0.29	26.20-26.43	26.47±3.75[†]
Globulin (g/L)	46.11±3.00	44.97-47.25	45.04±3.16	43.83-46.24	44.41±5.85[†]
Creatinine (μM)	38.03±1.66	37.39-38.66	39.19±1.66	38.55-39.82	31.19±14.54[†]
Total protein (g/L)	72.36±3.22	71.13-73.59	71.44±3.26	70.20-72.68	73.77±7.39[†]
Mineral-electrolyte profile					
Calcium ion (mM)	3.24±0.18	3.17-3.31	3.26±0.15	3.21-3.32	3.26±0.13[¥]
Inorganic phosphate (P_i)	1.70±0.27	1.60-1.81	1.62±0.20	1.55-1.70	1.61±0.20[¥]
Sodium ion (mM)	140.19±2.05	139.93-140.46	140.64±3.68	139.24-142.04	141.07±1.95[¥]
Potassium ion (mM)	4.37±0.53	4.16-4.57	4.34±0.62	4.10-4.57	4.33±0.40[†]
Magnesium ion (mM)	1.11±0.19	1.04-1.19	1.12±0.19	1.05-1.20	
Chloride ion (mM)	85.29±4.50	83.57-87.00	84.45±4.47	82.74-86.15	82.74±7.79[†]
Carbon dioxide (mM)	26.78±1.02	26.39-27.17	25.85±1.01	25.47-26.24	
Hydrogen carbonate (mM)	24.10±0.73	23.82-24.38	23.26±0.74	22.98-23.55	
Anion gap (mM)	35.16±6.91	32.53-37.79	37.26±7.80	34.30-40.23	
Enzyme profile					
Alanine aminotransferase (U/L)	87.81±1.99	87.05-88.57	88.70±1.24	88.22-89.17	89.07±3.72[†]
Alkaline phosphatase (U/L)	48.15±4.20	46.55-49.75	48.45±3.38	47.16-49.73	42.10±2.03[†]
Aspartate aminotransferase (U/L)	194.09±2.58	193.11-195.08	196.84±1.53	196.26-197.43	193.32±4.64[†]
Creatine kinase (U/L)	147.36±8.15	144.26-150.47	148.05±8.08	144.97-151.13	149.22±5.07[†]
γ-glutamyl transferase (U/L)	11.00±3.15	9.80-12.20	10.00±3.15	8.80-11.20	

Values are expressed as mean (x), standard deviation (SD), and as a confidence interval of 95% (CI); [†]*Alligator sinensis*, active period (Peng *et al.*, 2018); [¥]*Crocodylus palustris* (Stacy and Whitaker, 2000).

Quantified concentrations for serum biochemistry parameters are unprecedented at country level. Therefore, it was only possible to compare it with some parameters reported in *Alligator sinensis* (Peng *et al.*, 2018) and *Crocodylus palustris* (Stacy and Whitaker, 2000), but the differences were not statistically significant. Some differences in relation to sex were found (Table 3). The male crocodiles had significantly higher TAG and ALP activity, and significantly lower GLOB concentration than the females. The concentration of the other analytes did not show differences between sexes by species.

Meanwhile, differences in relation to species were found (Table 4). TB, TAG, CO_2 and HCO_3^- were higher in *Crocodylus moreletii* and BUN, Cre, ALT and AST were higher in *Crocodylus acutus*. The concentration of the other analytes did not show differences between species.

DISCUSSION

The health evaluations are very important for the protection of animal species in danger of extinction. Therefore, it is essential to establish reference ranges for serum biochemical profile, which could aid in the identification of alterations in animal metabolism. TAG and COL-T are often used as indexes in the assessment of nutritional status, and hyperlipidemia in obese animals (Kawasumi *et al.*, 2014). BUN and Cre are biochemical analytes related to each other, and very helpful to diagnose kidney pathologies in animals (Kaneko *et al.*, 2008). These conditions might well be associated, say because of a positive correlation (P<0.05) between BUN and Cre found in our study (Table 5). However, the concentration of TAG, COL-T, BUN, and Cre showed serum levels within the normal range. Our analysis thus suggests a correct protein and lipid metabolism.

The mineral-electrolyte profile also exhibited strong interionic interactions, which lead to strongly correlated movements of the ions. This implies directional correlations between the movements of distinct cations, between the movements of distinct anions, and between the movements of cations and anions (Iqbal *et al.*, 2019). Correlations can be positive, if distinct ions move preferentially into the same direction, e.g., between Ca^{2+}, P_i, Na^+, K^+, and Mg^{2+}. Or negative, if distinct ions move preferentially into opposite directions, e.g., between Cl^- with Ca^{2+}, P_i, K^+, and Mg^{2+}. These negative correlations exert a strong influence on the transport properties of the extracellular fluid e.g., Cl^- with A-gap (Figure 3). Like-ion correlations are generally stronger than cation-anion anticorrelations (Table 5) and are particularly strong for the heavier type of ion (Kim *et al.*, 2016).

CK typically presents in skeletal muscle, smooth muscle, heart, and brain. An increase CK activity is probably associated with skeletal muscle and cardiac muscle inflammation (Yousaf and Powell, 2012). As CK activity increases (Table 5), the level of serum TB also increases (Figure 4). Normally, the high level of TB concentration suggested hepatobiliary pathology (Imamura *et al.*, 2021). However, the level of serum CK and BT was quantified within the normal range, which suggests a correct muscle metabolism.

Table 3. Comparison of serum biochemistry parameters of male and female crocodiles sampled in Alcuzahue Lagoon of the Wildlife Management Unit; UMA-La Colorada., Colima, Mexico (*n* = 14 males and 15 females, per species)

	Crocodylus moreletii		*Crocodylus acutus*	
	Male	Female	Male	Female
	x ± SD			
Energy-lipid profile				
Glucose (mM)	3.93±0.52	3.86±0.43	3.92±0.34	4.10±0.51
Total cholesterol (mM)	6.30±0.68	5.97±0.85	5.74±0.71	6.12±0.70
Total bilirubin (μM)	1.23±0.09	1.25±0.09	1.18±0.07	1.19±0.05
Triacylglycerol (mM)*	9.19±0.19[a]	8.62±0.36[b]	7.88±0.07[a]	7.35±0.32[b]
β-hydroxybutyrate (mM)	0.86±0.14	0.84±0.16	0.81±0.21	0.77±0.18
Protein profile				
Blood urea nitrogen (mM)	0.47±0.03	0.46±0.03	0.52±0.01	0.53±0.01
Albumin (g/L)	26.29±0.32	26.20±0.32	26.35±0.24	26.35±0.34
Globulin (g/L)*	43.73±3.31[b]	48.33±2.66[a]	42.64±1.87[b]	47.99±1.36[a]
Creatinine (μM)	37.69±1.55	38.40±1.76	39.61±1.60	38.84±1.69
Total protein (g/L)	72.96±2.82	71.72±31.72	70.19±3.06	72.46±3.14
Mineral-electrolyte profile				
Calcium ion (mM)	3.28±0.16	3.20±0.20	3.21±0.13	3.31±0.15
Inorganic phosphate (P_i)	1.74±0.25	1.66±0.29	1.57±0.20	1.67±0.19
Sodium ion (mM)	140.41±1.74	139.95±2.38	139.31±2.86	141.72±4.00
Potassium ion (mM)	4.44±0.44	4.29±0.62	4.17±0.57	4.48±0.57
Magnesium ion (mM)	1.14±0.13	1.08±0.25	1.06±0.17	1.18±0.19
Chloride ion (mM)	84.51±3.94	86.12±5.06	85.37±4.65	83.70±4.33
Carbon dioxide (mM)	26.76±1.29	26.79±0.66	25.88±1.22	25.83±0.83
Hydrogen carbonate (mM)	24.09±0.92	24.12±0.50	23.30±0.89	23.24±0.63
Anion gap (mM)	36.25±5.89	34.00±7.92	34.81±7.00	39.26±8.00
Enzyme profile				
Alanine aminotransferase (U/L)	87.49±1.80	88.15±2.19	88.91±1.51	88.52±0.99
Alkaline phosphatase (U/L)*	51.42±4.47[a]	44.63±4.00[b]	51.14±1.53[a]	46.25±2.83[b]
Aspartate aminotransferase (U/L)	194.20±3.31	193.98±1.59	196.66±1.83	196.99±1.29
Creatine kinase (U/L)	146.06±6.42	148.76±9.74	146.33±9.71	149.45±6.48
γ-glutamyl transferase (U/L)	11.59±3.04	10.36±3.24	10.30±2.82	9.76±3.47

Values are expressed as mean (x) and standard deviation (SD); *Significant differences were obtained between groups indicated with different letters (P<0.05).

Table 4. Comparison of serum biochemistry parameters between species of crocodiles sampled in Alcuzahue Lagoon of the Wildlife Management Unit; UMA-La Colorada., Colima, Mexico (*n* = 29 *Crocodylus moreletii* and 29 *Crocodylus acutus*, respectively)

	Crocodylus moreletii	*Crocodylus acutus*
	x ± SD	
Energy-lipid profile		
Glucose (mM)	3.90±0.47	4.02±0.45
Total cholesterol (mM)	6.14±0.77	5.95±0.7
Total bilirubin (μM)*	1.24±0.08[a]	1.19±0.06[b]
Triacylglycerol (mM)*	8.92±0.40[a]	7.59±0.35[b]
β-hydroxybutyrate (mM)	0.85±0.14	0.79±0.19
Protein profile		
Blood urea nitrogen (mM)*	0.47±0.03[b]	0.52±0.01[a]
Albumin (g/L)	26.25±0.32	26.31±0.29
Globulin (g/L)	46.11±3.00	45.04±3.16
Creatinine (μM)*	38.03±1.66[b]	39.19±1.66[a]
Total protein (g/L)	72.36±3.22	71.44±3.26
Mineral-electrolyte profile		
Calcium ion (mM)	3.24±0.18	3.26±0.15
Inorganic phosphate (P_i)	1.70±0.27	1.62±0.20
Sodium ion (mM)	140.19±2.05	140.64±3.68
Potassium ion (mM)	4.37±0.53	4.34±0.62
Magnesium ion (mM)	1.11±0.19	1.12±0.19
Chloride ion (mM)	85.29±4.50	84.45±4.47
Carbon dioxide (mM)*	26.78±1.02[a]	25.85±1.01[b]
Hydrogen carbonate (mM)*	24.10±0.73[a]	23.26±0.74[b]
Anion gap (mM)	35.16±6.91	37.26±7.80
Enzyme profile		
Alanine aminotransferase (U/L)*	87.81±1.99[b]	88.70±1.24[a]
Alkaline phosphatase (U/L)	48.15±4.20	48.45±3.38
Aspartate aminotransferase (U/L)*	194.09±2.58[b]	196.84±1.53[a]
Creatine kinase (U/L)	147.36±8.15	148.05±8.08
γ-glutamyl transferase (U/L)	11.00±3.15	10.00±3.15

Table 5. Pearson correlation coefficients for serum biochemistry parameters of crocodiles sampled in Alcuzahue Lagoon of the Wildlife Management Unit; UMA-La Colorada., Colima, Mexico (*n* = 58 crocodiles)

	BUN[b]	GLOB[c]	Cre[d]	Ca^{2+}[e]	P*i*[f]	Na^{+}[g]	K^{+}[h]	Mg^{2+}[i]	Cl^{-}[j]	A-gap[k]	ALT[l]	ALP[m]	AST[n]	CK[o]
BT[a]	-0.05	0.05	0.09	-0.03	0.05	-0.04	0.03	0.01	0.09	0.04	0.26	0.25	0.26	0.71*
	BUN	0.22	0.46*	0.29	0.24	0.24	0.22	0.23	0.25	0.25	-0.01	-0.02	-0.01	0.20
		GLOB	0.26	0.17	0.20	0.22	0.25	0.22	-0.15	0.27	-0.02	0.67*	0.10	0.26
			Cre	-0.20	-0.24	0.25	0.20	0.23	0.23	0.27	-0.01	0.08	0.17	-0.03
				Ca^{2+}	0.81*	0.59*	0.80*	0.61*	-0.50*	0.79*	-0.03	-0.01	0.01	-0.01
					P*i*	0.40*	0.87*	0.68*	-0.56*	0.89*	0.01	0.04	0.15	-0.01
						Na^{+}	0.24	0.15	0.16	0.64*	-0.03	-0.02	-0.01	0.14
							K^{+}	0.65*	-0.54*	0.82*	-0.01	0.01	0.06	-0.02
								Mg^{2+}	-0.41*	0.51*	-0.11	-0.06	-0.11	-0.02
									Cl^{-}	-0.75*	0.01	0.03	0.10	-0.02
										A-gap	-0.01	0.03	0.07	-0.01
											ALT	0.25	0.67*	0.48*
												ALP	0.10	0.10
													AST	0.45*
														CK

[a]Total bilirubin; [b]Blood urea nitrogen; [c]Globulin; [d]Creatinine; [e]Calcium ion; [f]Inorganic phosphate; [g]Sodium ion; [h]Potassium ion; [i]Magnesium ion; [j]Chloride ion; [k]Anion gap; [l]Alanine aminotransferase; [m]Alkaline phosphatase; [n]Aspartate aminotransferase; and [o]Creatine kinase; *$P<0.05$.

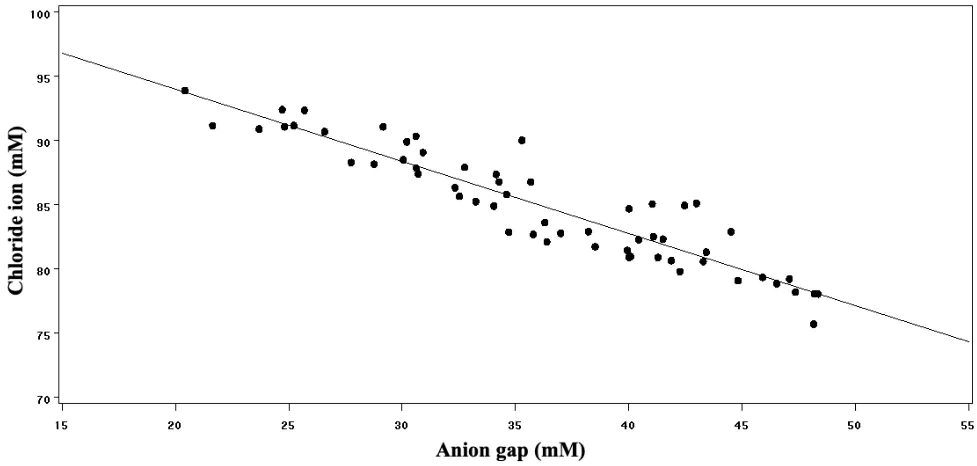

Figure 3. Relationship between chloride ion and anion gap. Chloride ion (●); predicted response (—).

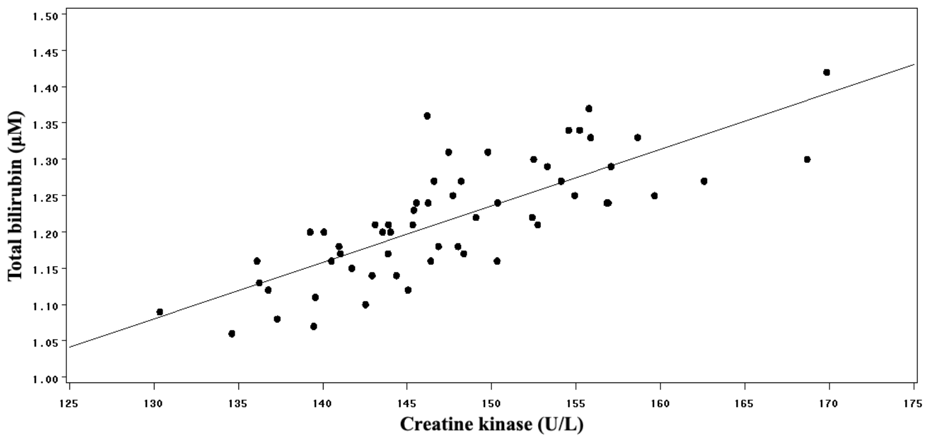

Figure 4. Relationship between total bilirubin and creatine kinase. Total bilirubin (●); predicted response (—).

ALP is a membrane-bound enzyme found in a wide variety of tissues, including liver and bone (Zender *et al.*, 2016). The level of serum ALP is used widely in the diagnosis of hepatobiliary pathology and various bone disorders (Ali *et al.*, 2006). ALP works at an alkaline pH and the difference in adipose tissue between sexes may influence its release from the liver into the circulation (Ali *et al.*, 2005). Therefore, the level of serum ALP of crocodiles could be influenced by the greater mass of total and abdominal adipose tissue of males compared to females (Table 3). Finally, AST is a liver-specific enzyme in humans and animals. The level of AST concentration in crocodiles is probably related to the enhancement of hepatic metabolism. Additionally, we found that the AST activity of *Crocodylus moreletii* and *Crocodylus acutus* was similar to *Alligator sinensis* in active period (Peng *et al.*, 2018), but greater than that of *Caiman crocodilus crocodilus* (Rossini *et al.*, 2011), and less than that of *Alligator mississippiensis* (Hamilton *et al.*, 2016). However, the concentration of ALT and ALP showed serum levels within the normal range. Our analysis thus suggests that liver function within this population has not been affected adversely by any current level of hepatic steatosis.

CONCLUSIONS

This report provided the first comprehensive reference ranges of serum biochemistry for captive bred *Crocodylus moreletii* and *Crocodylus acutus*. Most of the serum biochemistry parameters were similar among the sexes by species, only three significant differences existed in triacylglycerol, alkaline phosphatase, and globulin values. Some significant differences were found between species. Total bilirubin, triacylglycerol, carbon dioxide, and hydrogen carbonate were higher in *Crocodylus moreletii*, and urea nitrogen, creatinine, alanine aminotransferase, and aspartate aminotransferase were higher in *Crocodylus acutus*. These reference values are very important for the protection of crocodiles in danger of extinction. The calculated confidence intervals could be used to detect alert situations when at least 5% of the sampled crocodiles would fall outside of the calculated reference interval for a given parameter.

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