Physiological changes in serum glucidic and nitrogenic analytes from captive Argentine authoctonous caimans

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Abstract

Coppo, J.A.; Mussart, N.B.; Barboza, N.N.; Fioranelli, S.A.; Koza, G.A.; Prado, W.R.: ***Physiological changes in serum glucidic and nitrogenic analytes from captive Argentine authoctonous caimans.*** *Rev. vet. 17: 2, 103–108, 2006.* The purpose of this study was to establish reference intervals for serum glucidic and nitrogenic analytes from captive northeastern Argentinean caimans, as well as to detect physiological variations related to species, sex, age, feeding and season. Serum samples from 223 healthy sub–adults specimens of Caiman latirostris (n = 109) and Caiman yacare (n = 114), 50% each sex, were obtained. Values for glucose (4.18 ± 0.82 versus 4.01 ± 0.66 mmol/l), fructosamine (179 ± 33 versus 162 ± 29 umol/l), urea (1.11 ± 0.25 versus 1.06 ± 0.22 mmol/l), creatinine (56.1 ± 12.4 versus 52.5 ± 10.6 umol/l), and uric acid (127 ± 23 versus 156 ± 26 umol/l), were obtained by spectrophotometric methods for C. latirostris and C. yacare, respectively. Uric acid was significantly lower ($p < 0.05$) in C. latirostris. Glucose and urea were higher in females from both species. Progress of age (increment of liveweight and dimensions) correlated with increases of all biochemical parameters, significantly for glucose. Values were lower in winter, except for uric acid. The feeding system used in a hatchery resulted in glucose, urea and creatinine values higher than those registered in zoo specimens. Data obtained are applicable for caiman nutritional control, as well as for the diagnosis and prevention of diseases.

Key words: *Caiman latirostris*, *Caiman yacare*, glucose, fructosamine, urea, creatinine, uric acid, physiological changes.

Resumen

Coppo, J.A.; Mussart, N.B.; Barboza, N.N.; Fioranelli, S.A.; Koza, G.A.; Prado, W.R.: ***Cambios fisiológicos de los analitos glucídicos y nitrogenados en suero de caimanes autóctonos en cautiverio.*** *Rev. vet. 17: 2, 103–108, 2006.* El propósito del estudio fue establecer el intervalo de referencia para algunos analitos glucídicos y nitrogenados del suero de caimanes del nordeste argentino en cautiverio, así como detectar variaciones fisiológicas relacionadas a la especie, sexo, edad, alimentación y estación del año. Se tomaron muestras de 223 ejemplares sub–adultos sanos, de ambos sexos, de las especies *Caiman latirostris* (n = 109) y *Caiman yacare* (n = 114). Por espectrofotometría, en cada especie se obtuvieron respectivamente valores de glucosa (4,18 ± 0,82 versus 4,01 ± 0,66 mmol/l), fructosamina (179 ± 33 versus 162 ± 29 umol/l), urea (1,11 ± 0,25 versus 1,06 ± 0,22 mmol/l), creatinina (56,1 ± 12,4 versus 52,5 ± 10,6 umol/l) y ácido úrico (127 ± 23 versus 156 ± 26 umol/l). El ácido úrico fue significativamente más bajo ($p < 0,05$) en *C. latirostris*. Glucosa y urea fueron más altas en las hembras. El avance de la edad (incremento de peso vivo y dimensiones) causó aumento de todos los parámetros bioquímicos estudiados, significativamente para glucosa. Los valores fueron más bajos en invierno, excepto en el caso del ácido úrico. El sistema de alimentación usado en un criadero produjo valores más altos de glucosa, urea y creatinina que aquéllos registrados en especimenes de un zoológico. Los datos obtenidos son aplicables para el control nutricional, así como para el diagnóstico y prevención de enfermedades de los caimanes.

Palabras clave: *Caiman latirostris*, *Caiman yacare*, glucosa, fructosamina, urea, creatinina, ácido úrico, cambios fisiológicos.

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INTRODUCTION

Carbohydrate metabolism is explorable through glucose serum concentration, which reveals the glucemia "current value", and fructosamine serum level, wich indicates the glucemia "retrospective value" from the last two weeks [3]. When glucose and fructosamine are jointly considered, they give information about the energetic nutrition state, as well as the presence of malabsorption, stress, hepatopathy, hyper– and hypoadrenocorticism, hyper– and hypothyroidism, diabetes, and other diseases [1, 13].

Creatinine, urea and uric acid serum levels have utility to examine the non–protein nitrogen metabolism. Creatinine is the final residue of phosphocreatine, which is the main energy supplier for the muscular ATP resynthesis, whereas urea and uric acid are respectively terminal residues from protein and purine metabolism, although in birds urates can also derive from protids [8]. When these parameters act as metabolic indicators, they are able to reveal the functional state of liver and kidneys, the volume of muscular masses (growth), the intake of dietary nucleic acids and protein, and the nitrogen excretion pattern of the species [1, 3, 5].

In the same animal, nitrogen excretion pattern can register ontogenic changes related to water availability; some reptiles excrete ammonia when they stay into water, and uric acid or urea when they remain on land [11]. Non chelonid reptiles (vipers, lizards) are uricotelics, such as birds and insects; uric acid is the nitrogen metabolite that requires lowest quantity of water for its excretion. On the other hand, aquatic species are generally ammoniotelics because they have enough water for the nitrogen excretion. Animals that periodically leave the water detoxicate the ammonia convert into urea, whose excretion requires lower quantity of water than the amount necessary for the ammonia elimination. According to some authors, crocodiles would excrete their nitrogen as ammonia (75%) and uric acid [8, 24]. According to other [15], most of crocodile nitrogenous waste would be excreted as urate salts (70%), besides ammonia (25%) and urea (05%).

Some of these glucidic and nitrogenic analytes have been determined in northeastern Argentine authoctonous caimans, but studies were made on a lower sample number, with different techniques, and without establishing changes due to physiological variations [6, 20, 21]. The objective of the study was to obtain the reference interval for serum glucose, fructosamine, urea, creatinine and uric acid values from farmed *Caiman latirostris* and *Caiman yacare*, as well as to establish eventual differences related to the species, sex, age, feeding and year season.

MATERIAL AND METHODS

Experimental subjects. During 2 years, 223 caimans of "sub–adult" age [22], 50% of each sex, clinically healthy, from *C. latirostris* (n = 109) and *C. yacare* (n = 114) species, were studied. Health state was tested by exams of general state, dynamism, vivacity, appetite, skin and mucous coloration, and absence of external lesions. Some of them (n = 29) were maintained at the Corrientes City's Zoo, in small ponds, without roof, with running water, which was constantly renovated; they were fed with chicken viscera and fish. Remaining animals (n = 194) were housed at the "El Cachapé" farm (Chaco province), in roofed tanks with underground water, which was renewed daily and stayed at 27 ± 3ºC (heated by gas), fed with meat flour supplemented with vitamins and minerals; sporadically they received bovine viscera. To evaluate growth, sub–adult reptiles (1–5 years old approximately, 2–7 kg of liveweight, and 80–130 cm of length), were divided in 3 development stages, considering liveweight and body length, because is not possible to determine the crocodile age if hatching date is ignored [6].

Take of samples. Morphometrical and serological studies were made 4 times per year, in each season, in morning hours (8–9 am), after 12–hours of fast. Liveweight was obtained in a portable roman scale, and corporal dimensions were measured with a metallic metric tape. Blood extraction was carried out by venipunction of post–occipital venous sinus [10]; serum was separated by centrifugation.

Laboratory techniques. Serum determinations of glucose (oxydase–peroxydase method, measured at 505 nm), fructosamine (nitroblue tetrazolium, 530 nm), urea (urease, 570 nm), creatinine (alkaline picrate, 520 nm), and uric acid (uricase, 505 nm), were performed in a L.Mannheim 4010 UV–visible spectrophotometer, using Wiener Lab reagents [13]. Biochemical assays were tested by a quality control system, using liophilized comparison patterns (Standatrol).

Statistical analysis. Distributive normality was verified by Wilk–Shapiro test (WS). Parametric statistics included measures from central tendency (arithmetic mean, $\bar{x}$) and dispersion (standard deviation, SD). Fiduciary probability was evaluated by confidence intervals (CI±95%). Homogeneity of the variance was verified by Bartlett test. Analysis of variance (ANOVA) was made by one way linear model. Mean comparisons were carried out by Tukey test. Coefficient of linear association was evaluated by correlation (Pearson test). Calculations were made with the aid of a statistical software (*Statistix* 1996). For all inferences a 5% significance was specified, below which the equality null hypothesis was rejected [18].

RESULTS

Table 1 shows the biochemical values obtained for both reptile species jointly considered. The standard deviation of each arithmetic mean did not exceed the limit recommended by parametric statistic. WS coefficients

reveal that distribution was approximately normal. Confidence intervals were adjusted around means, but individual ranges were very wide.

Table 2 details the serum values registered for each studied species. Uric acid concentration was significantly higher in *C. yacare* than in *C. latirostris*. On the other hand, the last species showed lightly higher levels for remaining analytes.

Table 3 shows that all biochemical values, except for uric acid, were higher in females than in males, significantly for glucose and urea, in both grouped species. All studied analytes increased gradually when age progressed (increase of liveweight and dimensions), significantly in the case of glucose.

Table 4 reveals that environmental temperature (year seasons) caused significant differences for all the studied analytes. Serum concentration of glucose, fructosamine, urea and creatinine, were significantly higher in spring and summer, diminishing in autumn, and reaching their lowest values in winter. Blood level of uric acid was higher in the cold season. When the feeding systems from zoo and farm are compared, it is evident, except for uric acid, that nutrients supplied in the last produced higher serum concentrations of studied parameters; differences were statistically significant for glucose levels.

Pearson test revealed high linear association degree ($p < 0.05$) between liveweight and variables as total length ($r = 0.90$), muzzletail length ($r = 0.83$), head length ($r = 0.79$), head wide ($r = 0.86$), and thoracic perimeter ($r = 0.88$). Considering growth stages, liveweight correlated to serum glucose ($r = 0.95$; $p = 0.01$), fructosamine ($r = 0.79$; $p = 0.05$), urea ($r = 0.89$; $p = 0.03$), and creatinine ($r = 0.91$; $p = 0.01$). Increase of total length also correlated to the rise of same statistical variables.

Table 1: Values obtained in total studied population (n = 223).

parameter	$\bar{x} \pm SD$	WS	CI ± 95%	range
glucose (mmol/l)	4.12 ± 0.77	0.937	3.90 – 4.34	2.31 – 7.70
fructosamine (umol/l)	169 ± 31	0.976	151 – 187	60 – 280
urea (mmol/l)	1.09 ± 0.22	0.972	1.01 – 1.17	0.17 – 2.66
creatinine (umol/l)	54.3 ± 11.5	0.987	51.3 – 57.2	8.84 – 106.1
uric acid (umol/l)	139 ± 24	0.948	128 – 151	29 – 339

$\bar{x}$: arithmetic mean, SD: standard deviation, WS: Wilk–Shapiro distributive normality test (critical value: 0.947, $\alpha = 0.05$), CI±95%: confidence interval.

Table 2: Values obtained on each species.

parameter	*C. latirostris* (n = 109)		*C. yacare* (n = 114)	
	$\bar{x} \pm SD$	CI ± 95%	$\bar{x} \pm SD$	CI ± 95%
glucose (mmol/l)	4.18 ± 0.82	3.90 – 4.51	4.01 ± 0.66	3.79 – 4.29
fructosamine (umol/l)	179 ± 33	157 – 205	162 ± 29	128 – 183
urea (mmol/l)	1.11 ± 0.25	0.99 – 1.23	1.06 ± 0.22	0.97 – 1.16
creatinine (umol/l)	56.1 ± 12.4	52.1 – 60.2	52.5 ± 10.6	48.2 – 56.8
uric acid (umol/l)	127 ± 23 [a]	112 –141	156 ± 26 [b]	137 – 174

$\bar{x}$: arithmetic mean, SD: standard deviation, CI±95%: confidence interval. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

DISCUSSION

Eventual variations due to postprandial effect and circadian rhythm were excluded from the experimental design, because samples were taken during fast, in uniform morning hours. Nictameral endocrine and nervous variations produce changes in the blood concentration of certain analytes [3]. In crocodiles, lack of fast

Table 3: Variations according to sex, liveweight, and total length in both species ($\bar{x}$).

parameter	sex		liveweight (kg)			total length (cm)		
	male	female	< 3.5	3.5–5.0	> 5	< 100	100–110	> 110
glucose (mmol/l)	3.74 [a]	4.34 [b]	3.90 [a]	4.07 [a]	4.45 [b]	4.01 [a]	4.07 [a]	4.34 [b]
fructosamine (umol/l)	168	173	163	168	180	159	172	178
urea (mmol/l)	0.88 [a]	1.11 [b]	0.97	1.11	1.16	1.06	1.09	1.12
creatinine (umol/l)	50.5	55.4	53.1	55.2	57.3	52.7	54.4	56.8
uric acid (umol/l)	139	135	118	140	144	114	143	148

$\bar{x}$: arithmetic mean. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 4: Variations according to year season and feeding system in both species ($\bar{x}$).

parameter	year season				feeding	
	spring	summer	autumn	winter	farm	zoo
glucose (mmol/l)	4.40 [a]	5.00 [b]	3.57 [c]	2.97 [d]	4.45 [a]	4.07 [b]
fructosamine (umol/l)	185 [a]	192 [a]	166 [b]	159 [b]		
urea (mmol/l)	1.08 [a]	1.37 [b]	0.87 [c]	0.52 [d]	1.10	0.99
creatinine (umol/l)	67.7 [a]	73.9 [a]	47.9 [b]	41.6 [b]	59.4	56.1
uric acid (umol/l)	108 [a]	124 [a]	186 [b]	192 [b]	139	150

$\bar{x}$: arithmetic mean. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

causes hyperlipemia and interferes with photometric determinations [12].

Wide individual ranges verified in current study should be attributed to reptile physiological characteristics, because their blood values fluctuate considerably due to feeding system, environment temperature and sex [21]. Blood values from other aquatic animals, such as amphibians, also register greater oscillations due to their scarce regulation mechanisms, and to a higher tolerance to hemodilution and hemoconcentration [8].

For the comparison of biochemical values obtained here, with those found in the bibliography, in certain cases it was necessary to make a conversion from the Decimal Metric System to the International System of Units (SI) [13]. Values of serum fructosamine reported for crocodiles, were not found.

Glucose global serum values registered in the present study are similar to those obtained in *Alligator mississippiensis* (4.07 mmol/l) [19], although other authors report higher serum glucose concentration for this species: 5.88 ± 1.48 mmol/l [16]. Glucemia registered here do not greatly differ to those obtained in *Crocodylus niloticus* (4.50 mmol/l) [7]; for the same species, it was reported a higher plasma glucose rate (5.90 ± 0.90 mmol/l) [23]. Also higher is the concentration of the monosaccharide published for *Crocodylus acutus* (5.55 mmol/l) [19]. Ranges obtained for *Crocodylus porosus* are quite wider (4.50 to 12.10 mmol/l) [12] than those obtained here.

On *C. latirostris* farmed specimens (adults and juveniles, both sexes, n = 50), other authors communicate plasma glucose values (5.62 ± 0.57 mmol/l) [20] higher than the levels registered here in this species. In present study, glucemia was higher in *C. latirostris* than in *C. yacare*. Coincidently, on 34 captive caimans, other authors verify the same link between both species (4.73 mmol/l in *C. latirostris* and 4.55 mmol/l in *C. yacare*) [21]. On juvenile specimens from a hatchery, it was found also glucose higher values in *C. latirostris* (5.19 mmol/l) than in *C. yacare* (3.30 mmol/l) [6].

For *C. latirostris*, other authors report plasma urea values (1.14 ± 0.48 mmol/l) [20] similar to those of current study, which do not greatly differ from the averages obtained in captive *C. latirostris* (1.47 ± 0.06 mmol/l) and *C.yacare* (0.92 ± 0.16 mmol/l) [6]. In younger animals maintained in a hatchery, it was reported higher plasma urea concentrations on these species (1.62 and 2.02 mmol/l respectively) [21]. In agreement with the results from the last two assays, in current study urea serum levels were higher in *C. latirostris* than in *C. yacare*.

Serum uric acid levels from caimans investigated here, were higher than those obtained in *A.mississippiensis* (95.20 umol/l) [16], although they were similar to those found on the same species (170.5 umol/l) by another researcher [19], and lower than those reported on *C. niloticus* (410 umol/l) [7]. Individual ranges for uric acid from northeastern Argentine caimans were more narrow than those found in *C. porosus* (167 to 988 umol/l) [12]. Uricemias reported for *C. latirostris* and *C. yacare* (226 ± 14 and 187 ± 11 umol/l respectively) [6], as well as those published by others for the same species (231 ± 107 and 174 ± 26 umol/l respectively) [21], do not differ largely from those obtained in this trial. Nevertheless, in our caimans, serum level of uric acid was significantly higher in *C. yacare*, inversely to the rate described by those authors.

Creatinine confidence interval obtained here is more narrow than the range reported for *C. porosus* (20 to 51 umol/l) [12], and arithmetic means are higher than those published for *A. mississippiensis* (30 ± 9 umol/l) [16], and those informed for *C. latirostris* (32 umol/l) [20]. Creatinine averages obtained for *C. latirostris* and *C. yacare* (37 and 41 umol/l respectively [6], and 28 and 39 umol/l respectively [21]), are lower than those found in current study, where creatininemia was higher in *C. latirostris* than in *C. yacare*.

Biochemical values can be useful to evaluate caiman physiological state and to detect its illnesses on an early stage [21], but they must be correctly determined in the laboratory and must be compared with adequate reference intervals [3]. Several authors early quoted do not describe the techniques used in their works. Other investigators used dry chemistry methods, such as reactive strips. According to each case, heparinized plasma or serum were used. In certain studies, reptiles were not divided according to sex, age, or year season. Blood extraction techniques could be another cause of values variation [19], but they are not always specified in consulted references.

Sex–related significant variations registered here coincide with results obtained by other authors, who verified that sex causes marked differences in certain crocodile blood parameters; in *Crocodylus palustris* adult specimens, males revealed lower serum uric acid concentration than females [17].

Keeping in mind the increases of caimans liveweight and length, results indicate that growth produced elevation of all studied parameters, significantly for serum glucose concentration. Consecutive serum fructosamine raise, statistically correlated to glucemia ($r = 0.91$, $p = 0.02$), implies that monosaccharide increment was sustained along the time, and did not occur by hyperglucemic peaks, such as those caused by sympathetic alarms [1, 5]. This fact coincides with previous discoveries, which affirm that growth causes significant variations of certain blood analytes from *C. porosus* (yearlings versus 2 and 4 years old specimens) [2, 12].

Authors affirm that adult crocodiles have higher glucose plasma concentration than those in juvenile stage [17]. On the other hand, certain aquatic animals phylogenetically related to reptilian, show that growth causes decrease of serum glucose and uric acid concentrations, as well as increase of serum urea and creatinine levels, which are interpreted as resultants of the passage from juvenile ammoniotelic stage to adult ureotelic stage [4]. Serum creatinine increase registered here in caimans, could be related to the muscular masses increment, which happens during the growth [3], especially consid-

ering the high degree of linear association registered among this analyte, the liveweight, and the total length of studied specimens.

Lower serum levels of glucose and fructosamine registered in winter, can be attributed to the scarce energetic nutrients intake [1], just as it happens during crocodile hibernation [16]. In the same way, decrease of serum creatinine could be related to reduction of muscular activity during winter lethargy, and decrease of serum urea could be attributed to fall of dietary protein that happens when environmental temperature drops; increase of serum uric acid could be due to winter slowing down of hepatic excretion mechanisms [8]. During the hibernation of some amphibians (*Rana catesbeiana*), similar phenomena were corroborated [4]. States of malnutrition were verified in other crocodile species, as a consequence of winter depletion of tissular reserves [16]. Reaffirming the importance of environmental temperature, specimens of *C. latirostris* maintained at 22ºC registered higher liveweight gains than those housed at 18ºC [14].

Caimans housed in the hatchery registered higher serum concentration of glucose, urea and creatinine than those obtained on reptiles kept in the zoo, differences that are attributed to quantity, quality and frequency of the feeding. In current productive caiman rearing systems, feeding is the most important handling tools to be improved [6]. Standardization of clinical pathology techniques is important to appreciate the laboratory tests as effective tools on caiman breeding, meanly because ideal conditions for intensive crocodile rearing have not yet been established. Consequently, mortality is often directly linked to handling and nutritional failures [9].

In conclusion, physiological differences between species, as well as significant variations related to sex and age (liveweight and dimensions) from farmed *C. latirostris* and *C. yacare*, were verified. Marked fluctuations between different feeding types and year seasons were registered. It was detected that indicators related to nutritional state were lower in winter. A reference interval for glucidic and nitrogenic analytes, which was obtained from a numerous sample, by adequate laboratory techniques, tested by appropriate quality control methods, was established to be used in the diagnosis and metabolic and nutritional control of these reptiles.

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