REDVET Rev. electrón. vet. http://www.veterinaria.org/revistas/redvet
2012 Volumen 13 Nº 3 - http://www.veterinaria.org/revistas/redvet/n030312.html

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504

Medio interno en ejemplares juveniles de *Caiman latirostris* y *Caiman yacare* de Argentina. Variaciones fisiológicas según especie, sexo, peso, tamaño y estación del año - Internal environment in juvenile specimens of *Caiman latirostris* and *Caiman yacare* from Argentina. Physiological variations according to species, sex, liveweight, size, and season of the year

Noelia Nazaret Barboza, Norma Beatriz Mussart, Gabriela Alejandra Koza, José Antonio Coppo

Department of Physiology, Faculty of Veterinary Sciences, North-eastern National University UNNE, Sargento Cabral 2139, Corrientes (3400), Argentina. Tel. 03783-425753.

E-mail: fisiologia@vet.unne.edu.ar

Resumen

Los fluidos del medio interno, principalmente la sangre, son el reflejo del estado metabólico-nutricional del organismo. Para optimizar la cría de caimanes en cautiverio es necesario encontrar las dietas apropiadas para acelerar su crecimiento. Las dietas pueden evaluarse a través de las ganancias de peso, dimensiones corporales e indicadores nutricionales sanguíneos. El objetivo de esta investigación fue obtener valores de referencia y variaciones fisiológicas de los parámetros hematológicos y bioquímicos en ejemplares juveniles de *Caiman latirostris* y *Caiman yacare*. En un criadero del nordeste de Argentina se estudiaron 207 caimanes (50% de cada especie y sexo), los cuales fueron alimentados *ad libitum* con una mezcla de harina de carne y pellets balanceados (47 y 37% de proteínas respectivamente). Durante un año, en cada estación se efectuaron pesajes y mediciones de cinco dimensiones corporales y treinta y nueve analitos sanguíneos. Los resultados se procesaron por medio de análisis multivariado de la variancia (MANOVA), el cual reveló diferencias significativas entre especies y entre estaciones ($p<0,05$), pero no entre sexos. En promedio, albúmina, glucosa, calcio, magnesio, potasio, hemoglobina, HCM, CHCM, VCM, GGT, longitud total, longitud hocico-cloaca, ancho de cabeza, perímetro torácico y peso vivo, fueron más elevados en *C. latirostris*. En contraste, proteínas totales, globulinas, colesterol total, ácido úrico, triglicéridos, LDL-C, sodio, cobre, hematocrito, eritrocitos, leucocitos, CPK, ALP, AST, CHE, LDH y longitud de cabeza, fueron más altos en *C. yacare*. Los indicadores nutricionales revelaron valores más elevados en verano, circunstancia que se atribuye al cese de la alimentación de los caimanes durante el letargo invernal. La ausencia de variaciones intersexuales debe interpretarse teniendo en cuenta que los animales eran espécimenes jóvenes que aún no manifestaban conducta reproductiva. En conclusión, se

reportan valores de referencia para espécimenes sub-adultos de *C. latirostris* y *C. yacare*. El peso vivo, las medidas morfométricas y los valores de laboratorio variaron según la especie y estación del año. Se espera que estos conocimientos puedan ser aplicados para mejorar el sistema de cría de los caimanes.

Palabras claves: *Caiman latirostris*, *Caiman yacare*, peso vivo

Abstract

Internal environment fluids, mainly the blood, are the reflex of the metabolic-nutritional state of the organism. In order to optimize the captive breeding of caymans it is necessary to find appropriate diets to accelerate their growth. Diets can be evaluated through weight gains, body size and blood nutritional indicators. The objective of this assay was to obtain reference values and physiological variations of this parameters, in *Caiman latirostris* and *Caiman yacare* juvenile specimens. In a hatchery in northeastern Argentina, 207 caymans (50% of each species and sex), which were fed *ad libitum* with meat flour and balanced pellets (47 and 37% of protein respectively), were studied. Weighins and measurements of five corporal dimensions and thirty nine blood analytes, were carried out in each season during one year. Results were processed by means of multivariate analysis of the variance (MANOVA) and they showed significant differences between species and between seasons ($p<0.05$), but not between sexes. On average, albumin, glucose, calcium, magnesium, potassium, hemoglobin, MCH, MCHC, MCV, GGT, total length, muzzle-tail length, head width, thoracic perimeter and liveweight, were higher in *C. latirostris*. In contrast, total protein, globulin, uric acid, total cholesterol, triglycerides, LDL-C, sodium, copper, hematocrit, erythrocytes, leukocytes, CPK, ALP, AST, CHE, LDH and head length, were higher in *C. yacare*. Nutritional indicators revealed higher values in summer, circumstance attributed to the cessation of feeding during the caymans winter lethargy. The absence of intersexual variations should be interpreted keeping in mind that the animals were young specimens that still didn't manifest reproductive behavior. In conclusion, here are reported reference values for sub-adult category specimens of captive *C. latirostris* and *C. yacare*. Liveweight, morphometric sizes, and biochemical values varied according to species and season of the year. It is expected that this knowledge can be applied to improve the cayman breeding system.

Key words: *Caiman latirostris*, *Caiman yacare*, liveweight, corporal dimensions, blood values, physiological variations.

INTRODUCTION

Caiman latirostris and *Caiman yacare* (Figures 1 and 2) are two autochthonous species of crocodiles from the Alligatoridae family that inhabit the northeastern Argentina (Ferreyra and Uhart 2001). With the purpose of marketing the reptile leather and meat, hatcheries have recently proliferated in this area, they practice the ranching system. This procedure consists in inducing the hatching of eggs gathered from the natural environment, and rearing the caymans under controlled conditions until reaching commercial size for its slaughter and sale. The return to their environment of the dear percentage of animals that had survived under natural conditions is an important aspect of this exploitation system (Waller and Minucci 1993, Prado *et al*. 2001).

Figure 1. *Caiman latirostris*

Figure 2. *Caiman yacare*

In diverse places of the world, scientific investigation is directed to the objective of accelerating the captive caymans growth speed, to make it more profitable the production. This implies finding appropriate diets in quantity of food, quality of their components and digestibility of the nutritious principles (Piña and Larriera 2002). The use of blood nutritional indicators, joined with the evolution of liveweight and corporal dimensions, can cooperate to the achievement of this objective. Obtaining the reference range for laboratory values also assumes importance to optimize the diagnosis of illnesses of reptiles in captivity (Ferreyra and Uhart 2001, Uhart *et al.* 2001).

To obtain reference values for juvenile specimens (sub-adult category) from the species *C. latirostris* and *C. yacare* submitted to the same diet, as well as to verify differences attributable to sex and season (cold: autumn and winter, versus warm: spring and summer), were the objectives of the trial.

MATERIAL AND METHODS

Animals, facilities, food. A total of 207 clinically healthy caymans (104 *C. latirostris* and 103 *C. yacare*), approximately 50% of each sex (90 males and 117 females), were used. They were "sub-adults" animals, of 1-5 year-old, 2-7 kg liveweight, and 80-130 cm of total length. The caymans´ sex was determined *de visu* at the beginning of the assay, with the help of a speculum. The specimens were identified by means of caravans.

Reptiles were housed in the hatchery "El Cachapé" (Chaco Province, Argentina), in roofed tanks whose floor was 40% covered with subterranean water, which was renewed every other day. In winter, animals had heating (gas stoves and solar panels).

Caymans were fed *ad libitum* three times per week, with equal parts of meat flour (dry matter 92.98%; ash 24.42%; crude protein 47.17%; ether extract 13.41%; crude fiber 2.42%; nitrogen-free extract 3.04%; phosphorus 4.41%; calcium 5.05%), and balanced pellets (dry matter 92.95%; ash 8.79%; crude protein 37.52%; ether extract 4.52%; crude fiber 4.73%; nitrogen-free extract 44.34%; phosphorus 1.16%; calcium 0.8%).

Controls, sampling. Periodic controls were made four times, in each season of the year. Afterwards, data were separated in warm season (spring-summer) and cold season (autumn-winter). Weighings were made on a hanging scale and corporal dimensions mensurations were made with a metallic measuring tape, that is: total length (TL: from the muzzle to the end of the tail), muzzle-cloaca length (MCL: from the muzzle to the cloaca), head length (HL: from the muzzle to the occipital condyle), head width (HW: between the maxillary condyles) and thoracic perimeter (TP: at armpit level).

Blood was extracted with syringe and needle starting from the post-occipital venous sinus. An aliquot was treated with anticoagulant (EDTA) and the other one was centrifuged to obtain serum. These samples were preserved refrigerated (5ºC) until their processing in the laboratory.

Laboratory determinations. Hematological and biochemical tests were conceived in such a way that their spectrum embraced the exploration of both animal nutritional status and diverse organic functions feasible to be altered by illnesses (Coppo 2008). From the procedural point of view, spectrophotometry techniques were carried out in an L.Mannheim 4010 UV-visible apparatus. Biochemical determinations were made under an intralaboratory quality control system, using commercial comparison patterns (Standatrol). Flame photometry was made in a Metrolab 305-D apparatus with Biopur reagents. Electrophoretic migration

was carried out in a dish connected to an adjustable amperage power source Chemar CHF-I-3; ferograms were quantified in a Citocon CT-440 digital automatic densitometer, equipped with a printer.

Erythrogram: the hematocrit was evaluated by centrifugation of capillary tubes at 12,000 rpm. Concentration of red blood cells was obtained by means of microscopic counting in Neubauer hemocytometer, using specific reagents and methods for reptiles (Garcia *et al.* 1993, Campbell 1996). The hemoglobin determination was carried out by spectrophotometry, technique of the cyanmethaemoglobin, 540 nm wave longitude (WL), Wiener Lab reagents. The free nuclei of the hemolyzed erythrocytes were previously separate by centrifugation, according to avian method (Campbell 1996). Hematimetric indexes (mean corpuscular volume MCV, mean corpuscular hemoglobin MCH, and mean corpuscular hemoglobin concentration MCHC) were obtained by means of conventional calculations.

Leukogram: total leukocytes were evaluated by counting stained smear (indirect avian method, Biopur stain), and leukocytary formula (heterophils, lymphocytes, monocytes, eosinophils, and basophils) was performed by differential recount (200 cells) from smears stained according to May Grünwald-Giemsa technique, Biopur reagents.

Proteinogram: total protein were valued by spectrophotometry (biuret method, 540 nm WL, Wiener reagents). Seroprotein fractions (albumin and alpha, beta and gamma globulins) were separated by electrophoresis in cellulose acetate support, veronal-sodium buffer, amidoschwartz coloration, and ulterior quantification by densitometry. The albumin / globulins ratio (AGR) was obtained by calculation.

Non-protein nitrogen: the serum concentrations of urea (urease technique, 570 nm WL, Wiener reagents), creatinine (alkaline picrate method, 510 nm WL, Wiener reagents), and uric acid (uricase enzymatic technique, 505 nm WL, Wiener reagents), were determined by spectrophotometry.

Glucose: oxidase/peroxidase technique, spectrophotometry registration at 505 nm WL, Wiener reagents.

Lipidogram: it included determinations of triglycerides (glicerol-phosphate-oxidase / peroxidase technique, 505 nm WL, Wiener reagents), total cholesterol (cholesterol-oxidase-peroxidase, 505 nm WL, Wiener reagents), cholesterol bound to high density lipoproteins (HDL-C) and low density lipoproteins (LDL-C): by selective lipoprotein precipitation and enzymatic determination of the cholesterol (Wiener reagents), and alpha and beta lipoproteins (separation by electrophoresis in agarose gel,

veronal buffer, and Fat Red 7B coloration, Biopur reagents), quantifying by densitometry.

Ionogram: magnesium (xylidyl blue method, 510 nm WL, Wiener reagents), calcium (cresolphthalein complexone, 570 nm WL, Wiener reagents), inorganic phosphorous (molybdate-ascorbate technique, 620 nm WL, Wiener reagents), copper (bathocuproine method, 480 nm WL, Boehringer reagents), sodium and potassium (flame photometry with Biopur reagents).

Enzymogram: alkaline phosphatase ALP (phenylphosphate aminoantipyrine technique, 520 nm WL, 37ºC, Wiener reagents), gammaglutamyl transpeptidase GGT (glutamyl nitroanilide, 405 nm WL, 30ºC, Wiener reagents), creatinphosphokinase CPK (ATP-cysteine, 620 nm WL, 37ºC, Wiener reagents), lactate dehydrogenase LDH (NAD-lactate, 505 nm WL, 37ºC, Wiener reagents), aspartate aminotransferase AST, ex-GOT (aspartate-ketoglutarate, 505 nm WL, 37ºC, Wiener reagents), alanine aminotransferase ALT, ex-GPT (alanine-ketoglutarate, 505 nm WL, 37ºC, Wiener reagents) and butyrylcholinesterase CHE (butyryl thiocholine, 405 nm WL, 30ºC, Wiener reagents).

Statistical analysis. Data normal distribution was verified by the Shapiro-Wilk test. Quantitative variables were classified in ten groups: liveweight, morphometry, proteinogram, non protein nitrogen, glucose, lipidogram, ionogram, enzymogram, erythrogram and leukogram. An analysis of Pearson correlation was carried out to reduce the number of variables at the moment of carrying out the multivariate analysis.

It was made a matrix of data that included 32 biochemical and morphometric variables obtained in 207 specimens. An analysis of principal components (PC) was carried out with this matrix to obtain the arrangement of the species, sexes and seasons of the year (classification guideline), as well as to identify the variables that contributed to this ordination.

Starting from the most significant variables obtained from the analysis of PC, an multivariate analysis of the variance (MANOVA of two factors) was carried out, as well as a 5% Bonferroni test *a posteriori* to evaluate the differentiation grade between species and sex from experimental subjects. The total variability not due to differences between the groups (variability inside the groups) was evaluated by the Wilks' Lambda test. Statistica (StatSoft Inc. 2001, version 6) and InfoStat (Group InfoStat FCA, version 2008) were the informatic supports used for the statistical analyses.

RESULTS

Analysis of principal components. This test demonstrated that the first three PC expressed 80% of the total variance. The PC 1

constituted 41.1% of the variance and it was represented mainly by creatinine, potassium, calcium, MCH and MCHC, in a positive way, while AST and CPK, did it in a negative way. The PC 2 contributed with an additional 20.3% and it was represented positively by liveweight and morphometric parameters as HW and TP, as well as negatively by total cholesterol and hematocrit. The PC 3 gathered 18.7% of the total variance: total proteins contributed in positive way and copper, AGR, total leukocytes and eosinophils influenced in a negative way on this component (Table 1).

Table 1. Contribution of liveweight, morphometric and hematological variables to the first three principal components.

variable	PC 1	PC 2	PC 3
liveweight	-0.01	0.35	0.15
TL	-0.13	0.20	0.25
MCL	0.04	0.20	0.18
HL	-0.05	-0.22	0.19
HW	0.09	0.31	0.13
TP	0.04	0.34	0.07
total protein	-0.05	-0.17	0.35
AGR	0.15	0.14	-0.29
glucose	0.23	-0.08	0.21
total cholesterol	$3.4x10^{-3}$	-0.25	0.21
creatinine	0.25	-0.11	-0.03
uric acid	-0.23	-0.02	-0.17
sodium	0.12	0.12	0.10
potassium	0.25	$-1.3x10^{-3}$	0.14
calcium	0.26	-0.04	-0.06
inorganic phosphorous	0.22	-0.19	-0.07
magnesium	0.14	0.20	-0.12
copper	-0.16	0.07	-0.30
ALP	-0.23	0.16	0.09
ALT	0.10	0.13	-0.17
AST	-0.25	-0.15	-0.01
CPK	-0.24	-0.16	-0.01
hematocrit	0.11	-0.28	0.10
MCV	0.14	0.18	0.25
MCH	0.25	0.11	0.02
MCHC	0.24	0.03	-0.11
total leukocytes	0.11	-0.18	-0.28
lymphocytes	0.23	-0.12	0.11
eosinophils	0.10	-0.02	-0.32
heterophils	-0.23	0.04	-0.03
monocytes	-0.21	0.19	-0.11
basophils	0.20	0.09	-0.15
eingenvalues	13.15	6.50	6.00
accumulated variance (%)	41	61	80

PC: principal component, TL: total length, MCL: muzzle-cloaca length, HL: head length, HW: head wide, TP: thoracic perimeter, AGR: albumin/globulins ratio, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CPK: creatinphosphokinase, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

The two-dimensional graph that represents the ordination of species, sexes and seasons of the year obtained by analysis of PC (Figure 3) revealed the existence of four different groups formed by the two species of caymans and the two analyzed periods (warm and cold). Comparing the studied species it was evident that biochemical and morphological values were higher in *C. latirostris* than *C. yacare*. Within each studied species it was observed that the analyzed parameters were higher during the warm season than during the cold one. Within each species, the values of hematic and weight-height parameters of the males did not differ significantly from those of the females ($p<0.05$), except for *C. latirostris* in the warm season, during which females showed morphological values higher than males.

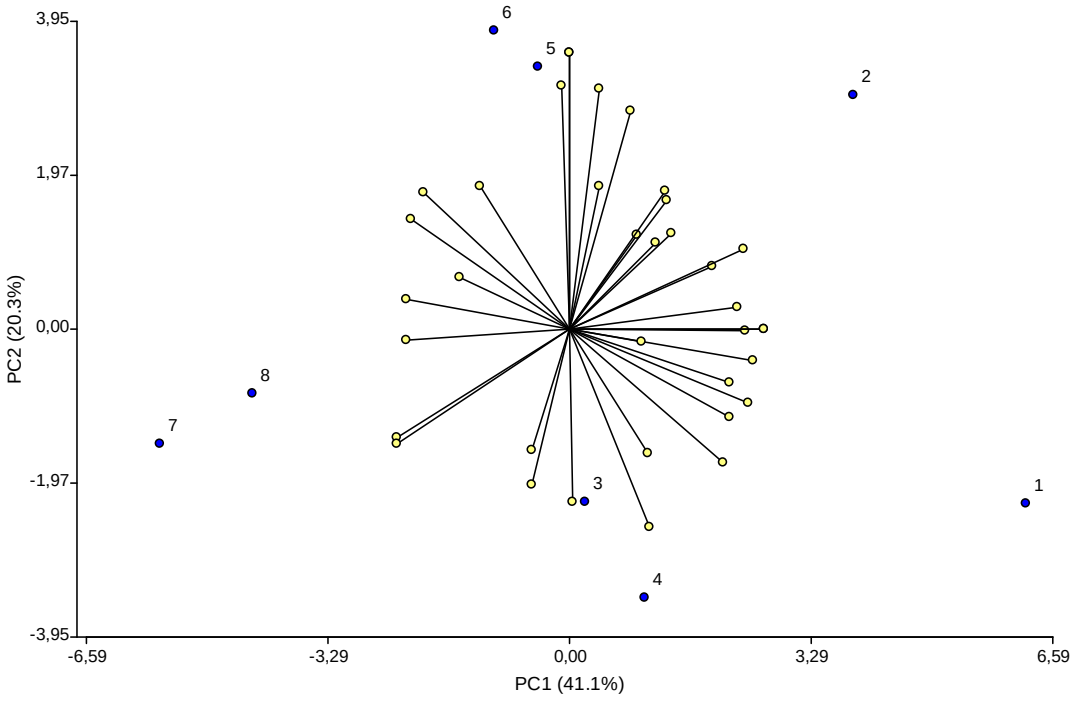

Figure 3. Analysis of principal components for the parameters liveweight, morphometry, and biochemical values. PC: principal component; 1: warm season, *C. latirostris* male; 2: warm season, *C. latirostris* female: 3: warm season, *C. yacare* male; 4: warm season, *C. yacare* female; 5: cold season, *C. latirostris* male; 6: cold season, *C. latirostris* female; 7: cold season, *C. yacare* male; 8: cold season, *C. yacare* female.

Multivariate analysis of the variance. The analysis of variance carried out inside the MANOVA for the effects species, sex and species by sex interaction (Table 2), revealed the existence of significant differences for liveweight and the most outstanding biochemical variables (defined by the previous analysis of PC) between the species *C. latirostris* and *C. yacare* (Wilks´ Lambda = 0.642, F = 6.108; p = 0.000). However, there were not significant differences between sexes (Wilks´ Lambda = 0.898, F

= 1.248; p = 0.241) nor for species x sex interaction (Wilks´ Lambda = 0.938, F = 0.720; p = 0.762).

Table 2. Analysis of the variance for the effects species, sex and their interaction.

variables	species		sex		species*sex	
	F	p	F	p	F	p
liveweight	5.367	0.022	0.687	0.408	3.108	0.080
total protein	6.866	0.010	2.496	0.116	1.411	0.237
AGR	13.132	0.000	3.296	0.071	1.818	0.179
glucose	0.230	0.632	3.943	0.049	3.270	0.072
total cholesterol	15.768	0.000	1.127	0.290	0.178	0.673
creatinine	1.374	0.243	0.287	0.593	0.193	0.661
uric acid	10.119	0.002	0.451	0.503	0.000	0.994
potassium	1.542	0.216	1.638	0.202	1.872	0.173
calcium	0.778	0.379	0.161	0.688	0.054	0.816
AST	20.543	0.000	0.013	0.908	0.000	0.994
CPK	11.932	0.001	0.593	0.442	0.077	0.782
MCV	1.868	0.173	1.815	0.180	0.599	0.440
MCH	9.905	0.002	4.594	0.033	0.626	0.430
MCHC	5.717	0.018	1.656	0.200	0.012	0.913
total leukocytes	0.066	0.798	0.037	0.848	0.267	0.606
Wilks' Lambda	6.108	0.000	1.248	0.241	0.720	0.762

AGR: albumin/globulins ratio, AST: aspartate aminotransferase, CPK: creatinphosphokinase, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

Liveweight and morphometry. In *C. latirostris*, liveweight and corporal dimensions were higher in the warm season than in the cold one. On the other hand, liveweight, total length, muzzle-cloaca length, and thoracic perimeter of *C. yacare* registered higher values in the cold season than in the warm one, contrarily to that observed for head length and head width (Table 3).

Table 3. Liveweight and morphometry according to season and species (X ± SD).

parameter	*C. latirostris*		*C. yacare*	
	warm season	cold season	warm season	cold season
liveweight (kg)	4.66 ± 1.15	4.37 ± 0.78	3.88 ± 1.15	3.93 ± 0.99
TL (cm)	104.48 ± 8.59	102.85 ± 5.89	101.11 ± 10.81	101.81 ± 8.67
MCL (cm)	51.07 ± 4.89	49.73 ± 3.17	49.21 ± 4.85	49.39 ± 4.35
HL (cm)	11.83 ± 0.87	11.46 ± 0.60	11.89 ± 1.04	11.73 ± 0.96
HW (cm)	8.05 ± 0.67	7.75 ± 0.51	7.59 ± 0.80	7.26 ± 0.66
TP (cm)	29.77 ± 3.12	28.62 ± 2.83	27.27 ± 3.07	27.33 ± 2.53

X: arithmetic mean, SD: standard deviation, TL: total length, MCL: muzzle-cloaca length, HL: head length, HW: head width, TP: thoracic perimeter.

Proteinogram. In *C. latirostris*, concentrations of total proteins and all globulins were higher in the warm season than in the cold one. On the

other hand, in *C. yacare* concentrations of total proteins, albumins, gamma-globulins and total globulins were higher in the warm season than in the cold one, when the levels of beta-globulins reached the highest values. The serum values of alpha-globulins and the albumins/globulins ratio did not register seasonal variations (Table 4).

Table 4. Proteinogram according to season and species (X ± SD).

parameter	*C. latirostris*		*C. yacare*	
	warm season	cold season	warm season	cold season
total protein (g/dl)	4.01 ± 0.62	3.68 ± 0.57	4.20 ± 0.53	3.90 ± 0.59
albumin (g/dl)	0.98 ± 0.27	1.00 ± 0.22	0.98 ± 0.20	0.92 ± 0.21
alpha globulin (g/dl)	0.77 ± 0.23	0.74 ± 0.19	0.73 ± 0.15	0.73 ± 0.18
beta globulin (g/dl)	0.85 ± 0.25	0.78 ± 0.18	0.92 ± 0.17	0.97 ± 0.18
gamma globulin (g/dl)	1.42 ± 0.32	1.18 ± 0.29	1.55 ± 0.35	1.29 ± 0.37
total globulins (g/dl)	3.03 ± 0.57	2.70 ± 0.47	3.20 ± 0.46	2.99 ± 0.54
albumin/globulins ratio	0.32 ± 0.09	0.37 ± 0.09	0.31 ± 0.07	0.31 ± 0.09

Non-protein nitrogen. Both in *C. latirostris* and in *C. yacare*, serum concentrations of urea and creatinine were lower in the cold season, while the values of uric acid were higher in the same time (Table 5).

Table 5. Non-protein nitrogen according to season and species (X ± SD).

parameter	*C. latirostris*		*C. yacare*	
	warm season	cold season	warm season	cold season
urea (mg/l)	84.9 ± 33.3	43.0 ± 29.8	76.7 ± 34.0	51.7 ± 29.7
creatinine (mg/l)	7.4 ± 2.2	5.1 ± 2.0	7.3 ± 3.2	5.6 ± 2.4
uric acid (mg/l)	17.2 ± 15.3	29.1 ± 14.2	20.5 ± 13.0	55.4 ± 25.4

Glucose and lipidogram. Blood levels of glucose, total cholesterol, triglycerides, HDL-C and beta lipoprotein, were higher in the warm season in both studied species, while LDL-C and lipoprotein alpha were higher in the cold time (Table 6).

Table 6. Glucose and lipidogram according to season and species (X ± SD).

parameter	*C. latirostris*		*C. yacare*	
	warm season	cold season	warm season	cold season
glucose (g/l)	0.91 ± 0.27	0.52 ± 0.10	0.79 ± 0.24	0.50 ± 0.17
total cholesterol (g/l)	0.34 ± 0.17	0.24 ± 0.13	0.55 ± 0.19	0.31 ± 0.13
triglycerides(g/l)	0.66 ± 0.48	0.30 ± 0.21	0.81 ± 0.51	0.33 ± 0.22
HDL-C (g/l)	0.03 ± 0.03	0.02 ± 0.01	0.06 ± 0.05	0.02 ± 0.01
LDL-C (g/l)	0.10 ± 0.08	0.16 ± 0.09	0.17 ± 0.12	0.18 ± 0.09
alfa lipoprotein (%)	82 ± 6	88 ± 5	82 ± 5	85 ± 7
beta lipoprotein (%)	18 ± 6	12 ± 5	18 ± 5	15 ± 7

HDL-C and LDL-C: cholesterol bound to high and low density lipoproteins respectively.

Ionogram. Serum values of sodium, potassium, calcium and inorganic phosphorous in *C. latirostris* were higher in the warm season, while magnesium and copper were higher in the cold time. On the other hand, only this last electrolyte registered higher winter concentrations in *C. yacare* (Table 7).

Table 7. Ionogram according to season and species (X ± SD).

parameter	*C. latirostris*		*C. yacare*	
	warm season	cold season	warm season	cold season
sodium (mEq/l)	150 ± 6	149 ± 7	152 ± 4	147 ± 9
potassium (mEq/l)	5.14 ± 0.56	4.36 ± 0.74	4.88 ± 0.58	3.97 ± 0.63
calcium (mg/dl)	9.00 ± 1.07	8.68 ± 0.98	8.92 ± 0.94	8.32 ± 1.32
phosphorous (mg/dl)	4.54 ± 1.52	3.72 ± 0.89	4.86 ± 1.26	3.73 ± 0.96
magnesium (mg/dl)	2.70 ± 0.28	2.76 ± 0.25	2.56 ± 0.33	2.45 ± 0.31
copper (ug/dl)	84 ± 36	131 ± 33	83 ± 36	156 ± 25

Enzymogram. *C. latirostris* presented serum activities of ALP, ALT, AST, GGT and CPK higher in the cold season; on the other hand, LDH and CHE were higher in the warm season. *C. yacare* showed higher activities of ALP, AST and CPK in the winter time and the rest of the enzymes were superior in the summer time (Table 8).

Table 8. Enzymogram according to season and species (X ± SD).

parameter	*C. latirostris*		*C. yacare*	
	warm season	cold season	warm season	cold season
ALP (UI/l)	48 ± 26	56 ± 19	50 ± 24	57 ± 25
ALT (UI/l)	13 ± 6	14 ± 5	14 ± 7	12 ± 7
AST (UI/l)	53 ± 15	66 ± 20	82 ± 35	110 ± 47
GGT (UI/l)	10 ± 6	11 ± 5	9 ± 5	7 ± 6
CPK (UI/l)	113 ± 74	152 ± 116	184 ± 104	255 ± 134
LDH (UI/l)	356 ± 150	277 ± 156	425 ± 174	386 ± 177
CHE (UI/l)	555 ± 179	307 ± 163	653 ± 223	347 ± 151

ALP: alkaline phosphatase. ALT: alanine aminotransferase. AST: aspartate aminotransferase. GGT: gammaglutamyl transpeptidase. CPK: creatinphosphokinase. LDH: lactate dehydrogenase. CHE: butyrylcholinesterase.

Erythrogram. In both species studied here, the parameters of erythrogram showed higher values in the warm season than in the cold one, except for the red blood cells concentration and MCHC, which did not register seasonal variations in *C. yacare* (Table 9).

Table 9. Erythrogram according to season and species (X ± SD).

parameter	*C. latirostris*		*C. yacare*	
	warm season	cold season	warm season	cold season
hematocrit (%)	21 ± 4	19 ± 3	21 ± 3	20 ± 4
erythrocytes (T/l)	0.47 ± 0.10	0.46 ± 0.10	0.51 ± 0.10	0.51 ± 0.10
MCV (fl)	442 ± 44	425 ± 42	429 ± 44	405 ± 36
hemoglobin (g/dl)	6.22 ± 1.42	5.58 ± 1.28	5.87 ± 1.48	5.52 ± 1.39
MCH (pg)	133 ± 23	122 ± 16	116 ± 22	109 ± 18
MCHC (%)	30 ± 5	28 ± 4	27 ± 5	27 ± 3

MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. MCHC: mean corpuscular hemoglobin concentration.

Leukogram. Total recounts of white blood cells and rates of heterophils and monocytes of both caymans studied here, were lower in warm season, during which the lymphocytes percentage was higher. No variations of eosinophils nor basophils rates were registered (Table 10).

Table 10. Leukogram according to season and species (X ± SD).

parameter	*C. latirostris*		*C. yacare*	
	warm season	cold season	warm season	cold season
leukocytes (G/l)	13.2 ± 3.8	14.2 ± 4.6	13.4 ± 3.3	14.8 ± 5.6
lymphocytes (%)	83 ± 7	65 ± 11	76 ± 9	68 ± 11
eosinophils (%)	2 ± 1	2 ± 1	2 ± 1	2 ± 1
heterophils (%)	11 ± 6	25 ± 10	18 ± 8	23 ± 11
monocytes (%)	4 ± 2	6 ± 4	4 ± 2	7 ± 3
basophils (%)	1 ± 1	1 ± 1	0 ± 0	0 ± 0

DISCUSSION

Liveweight and morphometry. In order to compare liveweights and morphometrical data obtained here, it should be mentioned that our autochthonous caymans are classified in four categories of biological importance according to their total length: I (0.23-0.40 m: younger than one year), II (0.41-1.30 m: sub-adults), III (1.31-1.70 m: male and female adult reproducers) and IV (> 1.70 m: adult males) (Waller and Minucci 1993). Total lengths obtained in the present study for sub-adults specimens of *C. latirostris* (1.04 m) and *C. yacare* (1.01 m) remained inside this referential mark.

Specimens of *C. latirostris* (2-4 year-old, n = 160) maintained in a hatchery of Santa Fe (Argentina), registered total length (40-103 cm) whose minimum range was significantly inferior to the registered in the present study for the group of both species (ξ = 80 cm) (Tourn *et al*. 1993). Comparing the values registered here with those reported for Australian saltwater crocodile (*Crocodylus porosus*), it arises that the growth of the latter would be more accelerated because yearling

specimens housed in hatcheries (n = 120) revealed maximum ranges of 7.25 kg of liveweight and 125 cm of total length (Millan *et al.* 1997).

When the newborn emerges from an egg, hatchlings of *C. yacare* are heavier than those of *C. lastirostris* (47.7 versus 45.0 g); they also reveal bigger total length (242 versus 230 mm), muzzle-cloaca length (117 versus 108 mm), head length (35 versus 32 mm) and head width (20.6 versus 20.2 mm). However, in experiences carried out in hatcheries in northeastern Argentina, it was proved that at the end of the first year, *C. latirostris* reaches a liveweight gain (1.44 g/animal/day) higher than *C. yacare* (0.75 g/animal/day), as well as bigger increase in total length (667 versus 591 mm) (Prado *et al.* 2001), changes that happened in the same direction as that registered in the present essay.

In spite of the fact that *C. yacare* specimens would be more sensitive to cold (Prado *et al.* 2001), in the present experience they revealed winter liveweights higher than summer ones (difference: +50 g). On the contrary, *C. latirostris* showed an opposite change (difference: -290 g). It is broadly admitted that during cold seasons, caymans interrupt or diminish the taking of food ("winter lethargy") and restrict their metabolism until a "saving phase" (Hoar 1983), which affects negatively liveweight gain because the sub-feeding of ectothermic species causes exhaustion of energy reserves (glycogen, lipids) and consumption of structural proteins (muscles), with stunting (Machado *et al.* 1988). In the same direction, a lot of *C. latirostris* maintained during two months at high environmental temperature (22ºC) revealed higher liveweight gains than controls maintained at lower temperature (18ºC) (Piña and Larriera 2002).

Proteinogram. Serum values of total proteins and total globulins higher in captive *C. latirostris* than in captive *C. yacare* (5.76 versus 5.61 g/dl; 3.07 versus 2.30 g/dl respectively) were obtained by others investigators in northeasterm Argentina; on the other hand, this last species registered higher values of albumins (2.58 versus 2.31 g/dl) (Troiano *et al.* 1997). The levels of total proteins and albumins registered in the present work were significantly lower than those mentioned *ut supra*, maybe due to the different methodology used for their assessment. The increments of proteins and total globulins registered here in the warm season can be attributed to the restoration of feeding after the ceasing of the winter lethargy (Machado *et al.* 1988).

Other authors reported higher values of total globulins for *C. latirostris* in captivity than in free-living specimens (3.48 versus 3.37 g/dl), on the other hand, in these last ones they registered higher levels of albumins and total proteins (1.66 versus 1.17 g/dl and 5.06 versus 4.59 g/dl). Finally, they obtained higher concentrations of albumins and AGR in captive *C. yacare* than in wild specimens (0.95 g/dl and 0.29 versus 0.87 g/dl and 0.23 respectively) (Uhart *et al*. 2001).

In opposition to the absence of intersexual variations emergent of the present test, in other species *(Alligator mississippiensis)* were found lower values of total proteins in males (5.53-5.64 g/dl) than in females (5.70-6.20 g/dl) (Lance *et al.* 1983). In studies carried out on *Crocodylus niloticus* were reported values of total proteins (3.10 g/dl -Foggin 1987- and 3.07 g/dl -Swanepoel *et al.* 2000-) lower than those obtained here for our South American caymans.

Non-protein nitrogen. The highest levels registered in summer for the first two indicators of the nitrogen metabolism, maybe should be related to the increase of the protein intake (case of urea) and to the consequent increment of muscular masses (case of creatinine). On the other hand, the increases of uric acid in the cold season could be attributed to the reduction of hepatic and renal functions as a consequence of the restricted metabolic activity, which is characteristic of the winter lethargy (Coppo 2008).

Uric acid is the main nitrogen metabolism residue of reptiles; its low toxicity allows its elimination in form of crystals by urine in the face of shortage of water, environmental condition to which the caymans are adapted (Eckert 1992). Contradictorily to the results obtained here, in hatchery caymans other authors verified that in summer the values of urea were higher in *C. yacare* than in *C. latirostris* (122 versus 97.5 mg/l respectively); on the other hand, during the warm season *C. latirostris* beat *C. yacare* levels of creatinine and uric acid (3.4 and 41.3 mg/l versus 3.2 and 29.4 mg/l respectively). In the same publication, higher summery values of urea and uric acid were reported for both species in free life (90 and 137 mg/l respectively), as well as lower levels of creatinine (4.5 and 3.5 mg/l), in relation to those mentioned here for the warm season (Uhart *et al.* 2001).

Wild *C. latirostris* specimens registered non protein nitrogen levels higher than those of *C. yacare* (urea: 62.6 versus 51.2 mg/l; creatinine: 3.7 versus 3.5 mg/l; uric acid: 35 versus 22 mg/l respectively) (Ferreyra and Uhart 2001). Captive *C. latirostris* females showed creatinine serum values of 1.8 mg/l, considerably lower than those obtained here (Trossero 2005). Captive *A. mississippiensis* specimens, related to the caymans studied in this trial, showed serum uric acid values of 30 mg/l (Barnett 1998), similar to those obtained here for *C. yacare*.

Glucose and lipidogram. Glucose and triglycerides serum concentrations in both species of caymans from the present essay, were lower in the cold season than in the warm one; such a situation was predictable due to the food intake decrease (Coppo 2008). The reptile alimentary behavior seems to be subjected to a circannual rhythm since it even happens in animals maintained at controlled environmental

temperature (Huchzermeyer 2003), like it is the case of the caymans studied here.

Innversely to the results of the present work, other authors found in captive *C. latirostris* higher total cholesterol concentration in the cold season than in the warm one (3.0 versus 1.5 g/l), even though the levels of HDL-C were higher in the warm season (0.61 versus 0.36 g/l) and those of LDL-C were higher in the cold season (1.76 versus 0.60 g/l), in agreement with results registered here from studied caymans (Tourn *et al.* 1993).

In coincidence with our results, another trial carried out on captive caymans reported that the values of serum glucose were higher in *C. latirostris* than in *C. yacare* (0.98 versus 0.77 g/l) and concentrations of total cholesterol were higher in *C. yacare* than in *C. latirostris* (0.79 versus 0.68 g/l). In contrast, in that essay were found lower levels of triglycerides in *C. yacare* than in *C. latirostris* (1.45 versus 1.98 g/l) (Troiano 1997).

Glucose values similar to those found here for *C. yacare* in warm season, were registered in *A. mississippiensis* (0.73 g/l) (Stein 1996). In this alligator, other investigator reported that glucemias of wild males and females were similar to each other (1.23 and 1.21 g/l respectively), but markedly higher than those found in captive animals (0.73 and 0.77 g/l respectively) (Lance *et al.* 2001). These last ones were similar to those registered in present essay.

***Ionogram*.** During the present experience, highest serum concentrations of most of the studied electrolytes in the spring-summer period maybe responds to food ingestion increment, like it is described for other species (Machado *et al.* 1988). In northeastern Argentina, other studies demonstrated that during the summer, captive specimens of *C. latirostris* and *C. yacare* showed similar serum values of sodium (150 and 148 meq/l respectively) and potassium (4.43 and 4.58 meq/l), but they registered considerably higher concentrations of calcium (13.51 and 10.54 mg/dl) and inorganic phosphorous (7.78 and 5.16 mg/dl respectively) (Uhart *et al.* 2001). Such differences could be attributed to food type or different laboratory technique for the determination (Coppo 2008).

In other works were reported higher values of calcium and inorganic phosphorous in *C. latirostris* than in *C. yacare* (9.63 versus 7.03 mg/dl and 5.08 versus 4.27 mg/dl respectively); this last species registered higher values of sodium and potassium than the first one (114 versus 109 meq/l and 4.38 versus 4.02 meq/l respectively) (Troiano *et al.* 1997). Sodium, potassium, calcium and inorganic phosphorous blood levels in captive specimens of *C. latirostris* were higher than in *C. yacare* (144.06 meq/l; 4.6 meq/l; 8.9 mg/dl and 5.64 mg/dl versus 129.33 meq/l; 3.51

meq/l; 5.86 mg/dl and 3.58 mg/dl respectively) (Ferreyra and Uhart 2001). In this essay, concentrations of potassium, calcium and magnesium were higher in *C. latirostris* than in *C. yacare*.

Wild *A. mississippiensis* male specimens showed higher levels of calcium and magnesium (12.48 and 3.21 mg/dl respectively) and lower concentration of copper (76 ug/dl) than those obtained here for Argentine caymans (Lance *et al.* 1983). Values mentioned *ut supra* were higher than levels of calcium and magnesium found by other investigators in wild specimens of the same species (11.48 and "25.75" mg/dl respectively) (Lance and Lauren 1984). We suppose that this last value was transcribed in error and it should be read "2.57" mg/dl.

***Enzymogram*.** In general, changes in enzymatic activities in the serum of reptiles should be interpreted in the same way as for mammals and birds (Campbell 1996). Increase of ALP usually indicates an elevated osteoblastic activity, but it can also happen by cholestasis. Elevations of ALT and AST would indicate hepatic or muscular damage. The enzyme GGT may originate in hepatic, pancreatic and renal tissues, therefore its plasmatic variation would be related to dysfunctions in these organs. CPK comes from the nervous tissue and from the skeletal and heart muscles. LDH is originated in liver and muscles, its increase could indicate tisular destructions. CHE comes from the liver and its decrease in plasm indicates insufficiency of this organ or intoxication for organophosphate compounds (Coppo 2008).

Captive specimens of *C. latirostris* registered higher serum levels of ALT (18 versus 14 UI/l), AST (135 versus 92 UI/l) and GGT (16 versus 15 UI/l) than *C. yacare*; on the other hand this last species revealed higher activities of ALP (105 versus 30 UI/l) and LDH (1911 versus 1348 UI/l) than *C. latirostris* (Troiano 1997). In relation to the enzymatic activities obtained in the present trial, other authors informed for *C. latirostris* and *C. yacare* in summer season, lower activities of ALP (24 and 18 UI/l respectively) and LDH (338 and 135 UI/l), as well as higher values of ALT (57 and 20 UI/l), AST (118 and 49 UI/l) and CPK (375 and 340 UI/l) (Uhart *et al.* 2001). Comparing the values obtained here for both species of caymans with those reported for captive mature females of *C. latirostris*, it arises that in these last ones the levels of CPK, LDH and CHE were lower (406; 3128 and 1056 UI/l respectively) (Trossero *et al.* 2005).

Erythrogram. Seasonal fluctuations of red series registered in this experience should be attributed to the nutritional status optimization, which is characteristic of the warm season, and its physiological detriment during the cold period. In this way, it has been reported that although these reptiles be maintained with the same quantity of food during the whole year, the low environmental temperature interferes in their

digestive process due to deficient secretion of enzymes in the alimentary tract (Troiano 1991).

In another investigation on erythrogram of captive *C. latirostris,* dissimilar data were obtained in relation to those registered here. In this sense, the summer and winter values were: hematocrit: 24 versus 22%, MCV: 455 versus 373 fl, hemoglobin: 11.6 versus 8.6 g/dl, MCH: 201 versus 167 pg, and MCHC: 53 versus 37% respectively (Troiano and Althaus 1993). Keeping in mind the average values registered between both species from present assay, other investigators found higher levels of hematocrit for *C. latirostris* and *C. yacare* in summer, both in captivity (24.8 and 22.17% respectively) and wild (24 and 23%) (Uhart *et al.* 2001).

Hematocrit values in captive specimens of both northeastern Argentina autochthonous caymans were investigated in other trials; the packed cell volume was higher in *C. latirostris* than in *C. yacare* (21.94 versus 18.41%); on the other hand in wild exemplars it happened the inverse situation because *C. yacare* registered higher values than *C. latirostris* (29.93 versus 22.97%) (Ferreyra and Uhart 2001). In another American species, *Caiman crocodilus*, higher ranges of hematocrit and erythrocytes were reported (20-30% and 0.50-0.64 T/l respectively). as well as hematimetric indexes (MCV: "42.64" fl; MCH: "11.39" pg; MCHC: 26.85%) (Rossini 2004) lower than those obtained in present rehearsal. Again, it seems to have been slipped another error in the original publication when writing some of the precedent values: we interpret that the MCV should be "426" fl and the MCH "113" pg.

Leukogram. It is asserted that white blood cells seasonal variations would be dependent on environmental temperature changes, photoperiod oscillations, and alimentary habits. In this way, leukocytary differences in juvenile specimens of both sexes *C. latirostris* were found between winter and autumn seasons; males registered higher values of total leukocytes in winter (10.00 versus 5.17 G/l) and females in autumn (14.50 versus 9.63 G/l). Also, both in autumn and in winter, mature males registered total leukocyte values (9.77 and 9.47 G/l, respectively) higher than mature females (9.67 and 8.61 G/l) (Garcia *et al.* 1993).

Specimens of *C. latirostris* from Santa Fe (Argentina) showed values of total leukocytes and heterophils higher in winter than in summer (24.36 versus 22.68 G/l and 5 versus 2% respectively); on the other hand lymphocytes percentage was higher in the warm season than in the cold one (68 versus 66%). Eosinophils, monocytes, and basophils didn't register seasonal differences (19; 5 and 1% respectively) (Troiano and Althaus 1997). Wild specimens of the Venezuelan cayman *C. crocodilus* showed lower values of total leukocytes and lymphocytes (10.4 G/l and 20% respectively) and higher rates of monocytes (7.8%) and

granulocytes (eosinophils: 15.0%; heterophils: 51.3%; basophils: 4.8%) (Rossini 2004) than those registered in present trial.

Captive *A. mississippiensis* exemplars from Florida (USA) revealed lower values of total leukocytes and lymphocytes (5.8 G/l and 11.1% respectively). as well as higher percentages of eosinophils (9.2%), heterophils (57.6%), basophils (1.2%) and monocytes (5.2%) than those obtained in the present investigation (Barnett *et al.* 1998). On the other hand, in wild specimens of the same species other authors found lower levels of total leukocytes (12.2 G/l) and lymphocytes (33%), as well as higher values of eosinophils (12%), monocytes (12%) and basophils (8%). For heterophils (considered as "neutrophils") it was registered a rate of 35%, which overcomes the percentage obtained in this work (Schoeb *et al.* 2002).

CONCLUSION

In conclusion, reference values are obtained for the juvenile stage (sub-adult category) of both species of autochthonous caymans from northeastern Argentina, maintained in captivity and fed with the same diet. Although they didn't register significant differences between sexes, it should be kept in mind that at this age the reproductive behavior is not yet manifested. Liveweight and morphometric dimensions varied according to species and season of the year. Multivariate statistics detected physiological differences between concentrations of blood components of *C. latirostris* and *C. yacare,* as well as changes attributable to the environmental temperature. The fact that most of metabolic-nutritional parameters were higher in the warm season allows to suppose a change in the metabolic activity magnitude of these ectothermal animals. Although they remained housed in enclosures protected from the bleakness and cold temperatures, these reptiles were not exempt of the influence of the photoperiod nor the "phylogenetic biological clock" detector of seasons of the year. It is expected that such knowledge can be applied to optimize the breeding system of autochthonous caymans.

Acknowledgement: for its collaboration in the English language revision, we thanks to María Soledad Troia Quirch and María Esperanza Quirch.

REFERENCES

1. **Barnett, J.D., Cardeilhac, P.T., Barr, B., Wolf, W., Bass, O.L., Fleming, D.M.** (1998): Utilization of thyroid hormone levels to determinate starvation in alligators from the Everglades National Park. *Proceeding International Association for Aquatic Animal Medicine* 29: 52-56.
2. **Campbell, T.W.** (1996): Clinical pathology. In: *Reptile medicine and surgery* (Mader DR Ed.). Saunders. Philadelphia. p. 248-257.

3. **Coppo, J.A.** (2008): *Fisiología comparada del medio interno*. EUCASA. Salta (Argentina). 309 p.
4. **Eckert, R., Randall, D., Augustine, G.** (1992): *Fisiología animal*. McGraw-Hill. Madrid. 795 p.
5. **Ferreyra, H., Uhart, M.** (2001): Evaluación y evolución del estado sanitario de *Caiman latirostris* y *Caiman yacare* en el Refugio El Cachapé. *Boletín Técnico de la Fundación Vida Silvestre Argentina* 55: 1-15.
6. **Foggin, C.M.** (1987): Diseases and disease control on crocodile farms in Zimbabwe. In: *Wildlife management: crocodiles and alligators* (Webb, G.J., Manolis, S.C., Whitehead, P.J. Ed.). Surrey Beatty & Sons. Chipping Norton (UK). p. 351-362.
7. **García, P.B., Matushima, E.R., Ramos, M.C., Dias, J.L., Verdade, L.M**. (1993): Variações sazonais do padrão hematológico e protéico de jacarés de papo amarelo (Caiman latirostris) em cativeiro. *Anais do III Workshop sobre Conservação e Manejo do jacaré do papo amarelo*. Piracicaba (São Paulo. Brasil). p. 51-60.
8. **Hoar, W.S.** (1983): *General and comparative physiology*. 3rd ed.. Prentice-Hall. New Jersey. 848 p.
9. **Huchzermeyer, F.W.** (2003): *Crocodiles: biology. husbandry and diseases*. CAB International. London. 337 p.
10. **Lance, V.A., Joanen, T., McNease, L.** (1983): Selenium, vitamin E, and trace elements in the plasma of wild and farm-reared alligators during the reproductive cycle. *Canadian Journal of Zoology* 61: 1744-1751.
11. **Lance, V.A., Lauren, D.** (1984): Circadian variation in plasma corticosterone in the american alligator *Alligator mississippiensis* and the effects of ACTH injections. *General and Comparative Endocrinology* 54: 1-7.
12. **Lance, V.A., Morici, L.A., Elsey, R.M., Lund, E.D., Place, A.R.** (2001): Hyperlipidemia and reproductive failure in captive-reared alligators: vitamin E, vitamin A, plasma lipids, fatty acids, and steroid hormones. *Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology* 128: 285-294.
13. **Machado, C.R., Garófalo, M.A., Roselino, J.E., Kettelhut, I.C., Migliorini, R.H.** (1988): Effects of starvation, refeeding and insulin on energy-linked metabolic processes in catfish (*Rhamdia hilarii*) adapted to a carbohydrate-rich diet. *General and Comparative Endocrinology* 71: 429-437.
14. **Millan, J.M., Janmaat, A., Richardson, K.C., Chambers, L.K., Fomiatti, K.R.** (1997): Reference ranges for biochemical and haematological values in farmed saltwater crocodile (*Crocodylus porosus*) yearlings. *Australian Veterinary Journal* 75: 814-817.
15. **Piña, C., Larriera, A.** (2002): Caiman latirostris growth: the effect of a management technique on the supplied temperature. *Aquaculture* 211: 387-392.
16. **Prado, W., Gómez, O., Balla, P.** (2001): Crianza en cautiverio y reintroducción de individuos. *Boletín Técnico de la Fundación Vida Silvestre Argentina* 55: 26-38.

17. **Rossini, V.M.** (2004): Determinación de los parámetros hematológicos de la baba (*Caiman crocodilus*) en hábitat silvestre. *Proceedings of XIX Panamerican Congress of Veterinary Sciences*. Buenos Aires. p. 214.
18. **Schoeb, T.R., Heaton-Jones, T.G., Clemmons, R.M., Carbonneau, D.A., Woodward, A.R., Shelton, D., Poppenga, R.H.** (2002): Clinical and necropsy findings associated with increased mortality among american alligators of lake Griffin. Florida. *Journal of Wildlife Disseases* 38: 320-337.
19. **Stein, G.** (1996): Hematologic and blood chemistry values in reptiles. In: *Reptile medicine and surgery* (Mader, D.R. Ed.). Saunders. Philadelphia. p. 118-152.
20. **Swanepoel, D., Boomker, J., Kriek, N.P.** (2000): Selected chemical parameters in the blood and metals in the organs of the Nile crocodile, *Crocodylus niloticus*, in the Kruger National Park. *Onderstepoort Journal of Veterinary Research* 67: 141-148.
21. **Tourn, S., Imhof, A., Costa, A., von Finck, C., Barriera, A.** (1993): Colecta de sangre y procesamiento de muestras en *Caiman latirostris*. *Proceedings of IV Workshop sobre Conservación y Manejo de Yacaré Overo, Caiman latirostris*. Santo Tomé (Santa Fe. Argentina). p. 25-30.
22. **Troiano, J.C.** (1991): *Manejo sanitario de reptiles en cautiverio*. Prensa Veterinaria Argentina. Buenos Aires. 176 p.
23. **Troiano, J.C., Althaus, R.** (1993): Hallazgos hematológicos en *Caiman latirostris* (Crocodylia-Alligatoridae) en condiciones de cautiverio. *Proceedings of IV Workshop sobre Conservación y Manejo de Yacaré Overo, Caiman latirostris*. Santo Tomé (Santa Fe. Argentina). p. 12-24.
24. **Troiano, J.C., Althaus, R.L., Malinskas, G.** (1997): Perfil bioquímico sanguíneo de las especies del género *Caiman* en condiciones de cautividad. *Revista Española de Herpetología* 11: 31-34.
25. **Trossero, S.M., Siroski, P., Piña, C.I.** (2005): Variación estacional del perfil bioquímico en hembras juveniles de *Caiman latirostris* criadas en cautiverio. *Proceedings Reunión Regional de América Latina y el Caribe del CSG/SSC/IUCN* (Santa Fe. Argentina). p. 220-233.
26. **Uhart, M., Prado, W., Beldoménico, P., Rossetti, C., Ferryera, M.C., Martinez, A., Bardón, J.C., Avilés, G., Karesh, W.** (2001): Estudios sanitarios comparativos de yacarés (*Caiman latirostris* y *Caiman yacare*) silvestres y cautivos. *Boletín Técnico Fundación Vida Silvestre Argentina* 55: 1-15.
27. **Waller, T., Minucci, P.A.** (1993): Relevamiento de la distribución, hábitat y abundancia de los crocodilios de la Provincia de Corrientes, Argentina. *Proceedings of Primera Reunión Regional del Grupo de Especialistas en Cocodrilos*. Santa Marta (Colombia). p. 341-385.