Serum enzymatic activities in captive northeastern–Argentina caymen (Crocodylia: Crocodylidae)*

Coppo, N.B.; Coppo, J.A.; Barboza, N.N.; Prado, W.S.

Cátedra de Fisiología General, Facultad de Ciencias Exactas, Químicas y Naturales, UNAM, Azara 1552, Posadas (3300), Argentina. E–mail: jcoppo@vet.unne.edu.ar

Abstract

Coppo, N.B.; Coppo, J.A.; Barboza, N.N.; Prado, W.S.: ***Serum enzymatic activities in captive northeastern–Argentina caymen (Crocodylia: Crocodylidae)****. Rev. vet. 16: 1, 16–20, 2005*. The aim of the study was to obtain reference values for some clinically useful enzymatic activities in serum of captive northeastern–Argentina caymen. Two hundred and twenty–three samples from *Caiman latirostris* (n = 109) and *Caiman yacare* (n = 114), both sexes sub–adults specimens (2–7 kg liveweight and 80–130 cm total longitude), were analyzed. Serum activities from ALP (55.1±7.4 IU/l), ALT (14.7±3.1 IU/l), AST (64.0±11.3 IU/l), GGT (8.9±1.6 IU/l), CPK (154±27 IU/l), LDH (353±67 IU/l) and CHE (359±70 IU/l), were determined by spectrophotometry. Significant physiological differences ($p < 0.05$) among species, sexes, ages and year season, were verified. As age advanced, ALP decrease was registered, which is explained by the osseous development decline. At the same time, AST, CPK, LDH and CHE progressively increased; this change is attributed to muscular masses ontogenic increase. Winter decreases of enzymatic values are imputed to metabolic fall caused by alimentary restriction. Obtained values can be useful for illnesses diagnosis and for metabolic and nutritional control of caymen.

Key words: *Caiman latirostris*, *Caiman yacare*, serum enzymes, reference interval, physiological variations.

Resumen

Coppo, N.B.; Coppo, J.A.; Barboza, N.N.; Prado, W.S.: ***Actividades enzimáticas séricas de caimanes en cautiverio en el nordeste argentino (Crocodylia: Crocodylidae)****. Rev. vet. 16: 1, 16–20, 2005*. El propósito del trabajo fue obtener valores de referencia para las principales actividades enzimáticas de uso clínico en sangre de caimanes del nordeste argentino sometidos a cautiverio. Fueron procesadas 223 muestras de *Caiman latirostris* (n = 109) y *Caiman yacare* (n = 114), sub–adultos de ambos sexos (2–7 kg de peso y 80–130 cm de longitud total). Por espectrofotometría se determinaron las actividades séricas de ALP (55,1±7,4 UI/l), ALT (14,7±3,1 UI/l), AST (64,0±11,3 UI/l), GGT (8,9±1,6 UI/l), CPK (154±27 UI/l), LDH (353±67 UI/l) y CHE (359±70 UI/l). Se constataron diferencias fisiológicas significativas ($p < 0,05$) entre especies, sexos, edades y épocas del año. El avance de la edad cursó con descensos de ALP, que se relacionan con la declinación del desarrollo óseo, así como con progresivas elevaciones de AST, CPK, LDH y CHE, que se atribuyen al aumento ontogénico de las masas musculares. La disminución invernal de los valores enzimáticos se imputa al descenso del metabolismo generado por la restricción alimentaria. Los valores obtenidos pueden resultar útiles para el diagnóstico de las enfermedades y el control metabólico–nutricional de los caimanes.

Palabras clave: *Caiman latirostris*, *Caiman yacare*, enzimas séricas, intervalo de referencia, variaciones fisiológicas.

INTRODUCTION

Caymen productive use can constitute an important benefit source to regional economies (leather, meat), and it would stimulate the adoption of appropriate controls for the conservation of species [14, 15]. "Yacaré overo" (*Caiman latirostris*) and "yacaré negro" (*Caiman yacare*) are autochthonous caymen from northeastern Argentina, whose population decreased by pillage until the year 1980 [8]. Later on, controls to facilitate their populational density increase were established, as the rearing systems based on eggs gathering and reptile partial return to their natural habitat [21].

In the *ranching system*, crocodile eggs are obtained from the natural habitat, and they were incubated indoor, under controlled conditions. Closed captivity rearing is carried out in farms until animals reach an appropriate

Recibido: 18 febrero 2005 / Aceptado: 6 abril 2005

* Proyecto 16Q267 (CIDET–UNAM).

size to be returned to their wild life with more survival possibilities; a part of them is sacrificed with productive purposes [2].

During captivity, transmissible, metabolic and nutritional illnesses can be suffered by reptiles, which can be prevented or diagnosed by laboratory tests, as serum enzymatic determinations [17]. Some data about enzymatic values in autochthonous caymen from Argentina northeastern have been investigated, but they were obtained on smaller quantity of specimens, and using analytical techniques different from the ones used in the present study, without considering the corresponding physiological variations [5, 19, 20].

Enzymes are catalytic proteins of great value for diagnosis due to the precocity of their alteration, rather than the specificity of their tissular origin [4]. Alkaline phosphatase plasmatic activity (ALP) principally comes from bones, although it is also originated in liver, that´s the reason why it rises in cholestasis, occupant masses, and hepatic abscesses. Physiologically it increases during bone development, and pathologically in osseous alterations, fractures, and vitamin D deficiency [1]. ALP can come from intestine and tumoral masses; it can also rise by gluco–corticoids induction [4, 10].

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were previously well–known as "transaminasas GPT and GOT"; they are originated in liver, skeletal muscle, heart, kidneys and nervous tissue. These enzymes increased in inflammatory, necrotic, and toxic processes from liver, heart, and skeletal muscular tissue. Also, they rise in cholestasis, cirrhosis, hepatic metastasis, acute pancreatitis, hemolysis, renal infections, dermato–myositis and salicylate administration. ALT decreases in B_6 vitamin deficiency [1, 10].

Gammaglutamyl transferase (GGT) is synthesized in liver, pancreas, and kidneys; this enzymatic activity rises in alterations from these organs, especially in obstructive jaundice, biliary cirrhosis, acute hepatitis, and liver and pancreas cancer. Creatine phosphokinase (CPK) comes mainly from heart and skeletal muscles, as well as from nervous tissue. Physiologically it is increased by physical exercise, and pathologically by muscular lesions and heart necrosis. Lactate dehydrogenase (LDH) is elaborated in liver, and skeletal and cardiac muscles. Their serum increase is related to tissular destructions, necrosis, traumatisms, inflammations, infections, neoplasias and hemolysis [1]. Other LDH iso–enzymes come from lungs, kidneys, pancreas and leukocytes [4]. The type of cholinesterase (CHE) evaluated in present trial (butyryl cholinesterase) is synthesized in liver, that´s why their plasmatic decrease reveals alterations from this organ, as hepatic insufficiency, viral hepatitis, and carbamate and organo–phosphorated compounds intoxications [1, 4, 10].

The aim of this study was to obtain reference intervals of main enzymatic activities for clinical use, in serum of captive *C. latirostris* and *C. yacare* specimens, as well as to establish eventual physiological variations attributable to species, sex, age, feeding and year season.

MATERIAL AND METHODS

Experimental subjects. During 2 years, 223 serum samples from clinically healthy crocodiles (109 *Caiman latirostris* and 114 *Caiman yacare*), approximately 50% each sex (104 males and 119 females), were obtained. They were *sub–adults* animals [21], from 1–5 year–old, 2–7 kg liveweight, and longitude of 80–130 cm. Most of crocodiles (n = 194), were housed in the hatchery "El Cachapé" (Chaco, Argentina), a private farm from the Program of Refuges of Fundación Vida Silvestre Argentina [2], in roofed tanks whose floor was 40% covered by water, which was renewed every other day. Three times a week reptiles were fed ad libitum on meat flour supplemented with vitamins and minerals; sporadically they received bovine viscera. In winter these animals had been heated (gas stoves and solar panels). Remaining caymen (n = 29) were maintained in the Zoo of Corrientes City, Argentina, in enclosed areas without roof, whose floor was 50% covered by water; they were fed on chicken viscera, fish, and –occasionally– bovine meat.

Taking of samples. Morphometrical studies and blood extractions were made 4 times per year (summer, autumn, winter and spring), in morning hours (8–9 AM), after 12 h fast, without using anesthetics or tranquilizers. Crocodiles were captured with a lasso, and their jaws were tied for security. Liveweight was obtained in a portable balance, and corporal dimensions were evaluated by a metallic measure tape. Blood extraction (5 ml) was carried out from the post–occipital veined sinus [9], with syringe and needle. Blood was centrifuged to obtain serum, which was kept cooled at 5ºC until its analysis, which was made before 3 hours post–extraction.

Laboratory techniques. Using a L.Mannheim 4010 UV spectrophotometer, in thermostatized quartz micro–tubes (10 mm light–hole), with Wiener Lab reagents, determinations of ALP (method of phenylphosphate aminoantipyrine, readings to 520 nm, 37ºC), ALT (alanine–ketoglutarate, 505 nm, 37ºC), AST (aspartate–ketoglutarate, 505 nm, 37ºC), GGT (glutamil nitroanilide, 405 nm, 30ºC), CPK (ATP–cysteine, 620 nm, 37ºC), LDH (NAD–lactate, 505 nm, 37ºC) and CHE (butyrilthiocholine kinetic method, 405 nm, 30ºC), were made [1, 10]. All biochemical determinations were carried out with an intra–laboratory quality control design, using reference patterns (*Standatrol*).

Experimental design and statistical analysis. A totally randomized design was used. Independent variables were species, sex, year season (climate), and origin place (feeding, handling). To evaluate development (age), reptiles were divided in 3 groups, in growing order of liveweight and corporal longitude. Dependent quantitative continuous variables were the enzymatic activity serum values. Normality of distribution was verified by test of Wilk–Shapiro (WS). Parametric statistics included measures of central tendency (arithmetic mean, $\bar{x}$)

and dispersion (standard deviation, SD). Fiduciary probability was evaluated by means of confidence intervals (CI±95%). Analysis of variance (ANOVA) was made by one way linear model. Variance homogeneity was estimated by Bartlett test. When ANOVA was significant ($p < 0.05$), arithmetic means were compared by Tukey test. Coefficient of lineal 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.

Table 1. Obtained values in total population (n = 223).

enzyme	$\bar{x} \pm SD$	WS	CI±95%	range
ALP (IU/l)	55.1 ± 7.4	0.944	52.4 – 57.7	30 – 98
ALT (IU/l)	14.7 ± 3.1	0.972	13.9 – 15.5	6 – 28
AST (IU/l)	64.0 ± 11.3	0.993	61.1 – 66.9	14 – 120
GGT (IU/l)	8.9 ± 1.6	0.923	7.8 – 10.1	3 – 19
CPK (IU/l)	154 ± 27	0.928	139 – 168	18 – 393
LDH (IU/l)	353 ± 67	0.984	321 – 386	46 – 675
CHE (IU/l)	359 ± 70	0.952	335 – 382	136 – 590

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

RESULTS

Global values obtained for both reptile species are exposed in Table 1. Coefficients reached by WS test reveal an approximately normal distribution. Data dispersion (SD) did not exceed the limits recommended by parametric statistics. Confidence intervals were adjusted around arithmetic means, but individual ranges were wide. Values reached by each studied species are shown in Table 2. All enzymatic activities, except GGT, were higher in *C. yacare* than in *C. latirostris*, significantly in AST case.

In group composed by both crocodile species, CPK and LDH serum values were significantly higher in males than in females (Table 3). When liveweight and corporal longitude increased (growth), ALP significant declines and AST, CPK, LDH and CHE gradual elevations, were registered.

Except for ALP case, remaining enzymatic activities were lower in cold seasons (autumn, winter) than in warm ones (spring, summer), significantly for ALT, AST, CPK, LDH and CHE (Table 4). In these cases, lowest values were registered in winter. On the other hand, no significant differences were verified between zoo reptiles versus hatchery ones, which contrasted by different feeding and handling types.

Pearson test revealed high linear association degree ($p < 0.05$) between liveweight and variables as total longitude ($r = 0.90$), muzzle–tail longitude ($r = 0.83$), head longitude ($r = 0.79$), head wide ($r = 0.86$), and thoracic perimeter ($r = 0.88$). Considering the ages groups, no significant correlations were registered among liveweight and enzymes as ALT, AST, GGT or CHE. On the other hand, significant linear associations between liveweight and activities of ALP (–0.94; $p = 0.01$), CPK (0.89; $p = 0.02$) and LDH (0.90; $p = 0.007$), were verified. A rise of total longitude also is correlated to ALP decreases, and CPK and LDH increases.

DISCUSSION

Eventual variations due to circadian rhythm and postprandial effect were excluded from experimental design, because samples were taken in uniform morning hours, previous fast. In crocodiles, the lack fast causes hyperlipemia and it interferes photometric determinations [13]. Admitting that quality control has assured accuracy and precision of results obtained in laboratory [1], wide individual ranges verified in this study should be attributed to reptilian physiologic particularities, whose blood values fluctuate considerably due to feeding system, habitat, climate and sex [20]. Blood values from other aquatic animals, as amphibians, also register greater oscillations due to their scarce regulation mechanisms, and to a higher tolerance to hemodilution and hemoconcentration [7].

Serum ALP values obtained in present study aren´t far away from the average published for African freshwater crocodile, *Crocodylus niloticus* (64 IU/l) [22]. The ALP range here registered for northeastern–Argentina freshwater caymen, matches approximately with the range reported for Australian saltwater crocodile, *Crocodylus porosus* (31–180 IU/l) [13]. On the other hand, ALP levels found in 12 captive juvenile *C. latirostris* (26±10 IU/l) and 5 *C. yacare* specimens (18±7 IU/l), as well as those registered in wild animals from both species (24±17 and 12±1 IU/l respectively), were lower [20].

ALT activity obtained in this trial is similar to the published for *C. niloticus* (13 IU/l) [6] and it is located in proximities from the lowest limit on the range obtained in *C. porosus* (11–51 IU/l) [13]. It also coincides approximately with the value registered in juvenile and adults specimens of *C. latirostris*, although dispersion was higher (19±14 IU/l) [19]. On a minor number of juvenile *C. latirostris* and *C. yacare* in captivity (72 cm longitude, 540–780 g liveweight), lightly higher ALT values (27±8 and 20±7 IU/l respectively), were found [20]. On the same species of juvenile captive reptiles (0.5–1.2 kg liveweight), by dry chemistry techniques, considerably higher levels of ALT were obtained (47±4 and 31±4 IU/l respectively), which didn't vary too much on free life specimens (47±3 and 26±2 IU/l) [5]. In two last investigations, ALT was higher in *C. latirostris* than in *C. yacare*, circumstance that could not be corroborated in this work, because they resulted similar.

AST value here registered in *C. latirostris* is evidently lower than the levels obtained in same species by other investigators: 163±114 IU/l [19], 103±35 IU/l [20] and 83,4±6,9 IU/l [5]. Values of this enzyme found in *C. yacare* in the last mentioned work (83,1±8,6 IU/l) were also higher, although other authors report lower concentrations of AST on this species (49±6 IU/l), which rise

Table 2. Obtained values according to species.

enzyme	*C. latirostris* (n = 109)		*C. yacare* (n = 114)	
	x̄ ± SD	CI±95%	x̄ ± SD	CI±95%
ALP (IU/l)	54.2 ± 7.2	50.6 – 57.7	55.8 ± 7.6	51.9 – 59.7
ALT (IU/l)	14.4 ± 3.0	13.5 – 15.4	15.1 ± 3.2	13.8 – 16.2
AST (IU/l)	56.9 ± 9.8 [a]	53.2 – 60.7	72.4 ± 12.6 [b]	68.4 – 76.5
GGT (IU/l)	10.6 ± 1.8 [a]	8.9 – 12.3	7.4 ± 1.2 [b]	6.1 – 8.7
CPK (IU/l)	124 ± 18	106 – 143	185 ± 29	164 – 207
LDH (IU/l)	332 ± 63	289 – 375	374 ± 70	325 – 424
CHE (IU/l)	350 ± 68	317 – 382	368 ± 71	334 – 403

x̄: arithmetic mean, SD: standard deviation, CI±95%: confidence interval. In each file, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 3. Variations according to sex, liveweight and total longitude in both species (x̄).

enzyme	sex		liveweight (kg)			total longitude (cm)		
	M	F	<3.5	3,6–5,0	>5	<99	100–110	>110
ALP (IU/l)	53.9	56.4	67.2[a]	54.5[b]	49.1[c]	65.8[a]	57.7[b]	48.3[c]
ALT (IU/l)	14.8	14.5	15.0	14.5	15.7	14.3	14.7	15.6
AST (IU/l)	65.9	63.2	57.5[a]	66.3[b]	67.9[b]	56.6[a]	64.9[b]	67.7[b]
GGT (IU/l)	8.7	9.2	7.9	9.6	8.5	7.8	9.9	8.1
CPK (IU/l)	169[a]	144[b]	129[a]	151[b]	177[c]	138[a]	151[b]	169[c]
LDH (IU/l)	362[a]	340[b]	292[a]	350[b]	413[c]	320[a]	336[a]	407[b]
CHE (IU/l)	351	363	331[a]	370[b]	372[b]	351[a]	358[a]	374[b]

x̄: arithmetic mean, M: male, F: female. In each file, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 4. Variations according to year season and feeding system in both species (x̄).

enzyme	year season				feeding	
	spring	summer	autumn	winter	hatchery	zoo
ALP (IU/l)	51.8	55.4	47.9	56.1	55.0	55.5
ALT (IU/l)	19.0 [a]	15.1 [b]	14.5 [b]	12.7 [c]	14.2	15.4
AST (IU/l)	72.4 [a]	72.7 [a]	61.2 [b]	58.9 [b]	65.2	63.4
GGT (IU/l)	9.3	9.1	8.4	8.6	9.2	8.7
CPK (IU/l)	152 [a]	183 [b]	149 [a]	137 [c]	155	152
LDH (IU/l)	421 [a]	403 [a]	313 [b]	302 [b]	361	349
CHE (IU/l)	423 [a]	396 [a]	326 [b]	319 [b]	373	358

x̄: arithmetic mean. In each file, different letters indicate significant differences (Tukey test, $p < 0.05$).

in wild animals (57±18 IU/l) [20]. *C. niloticus* would have values of AST as low as 17 IU/l [6] and *C. porosus* would register ranges as wide as 23–157 IU/l [13].

No data on crocodile GGT levels were encountered. CPK values found in captive *C. latirostris*, from 569±101 IU/l [5] and 420±289 IU/l [20], are considerably higher than those of the present trial. CPK levels registered by others in *C. yacare* are also higher: 340±95 IU/l [20] and 189±39 IU/l [5], eventhough the last value comes closer to arithmetic mean here found on this species. Serum LDH activities reported on *C. latirostris*, from 406±175 IU/l [20] and 480±79 IU/l [5], are not very different to those registered in the present study, but those published by the same authors for *C. yacare* were considerably lower (135±81 and 114±10 IU/l respectively), especially in free life specimens (58±27 and 61±7 IU/l respectively). The level of LDH reported in another work for *C. latirostris* (1020±321 IU/l) [19], is three times higher than the value here obtained on the same species.

Great variability among exposed values could be attributed to differences in the place of venepuncture, time of blood extraction, use of heparin, lack of previous fast, different age and sex, year season, sample dimension, delay in analytic procedures, and use of laboratory techniques different from those employed in this study, just as it is described [1, 10, 9]. Marked differences in certain crocodile hematic parameters would be conditioned by sex [18]. Here, CPK and LDH activities were significantly higher in males than in females, maybe because males possess more voluminous muscular masses, just as it happens in other species [4].

In the same sense, progressive AST, CPK, LDH and CHE elevations verified when age increased (liveweight, longitude), could be attributed to ontogenetic reasons, especially to muscular volume increase. Supporting this hypothesis, linear association coefficients verified among the increases of liveweight, total longitude and some of those enzymatic activities, were high. In coincidence, when data obtained on 1 year–old *C. porosus* [13], are compared to values reported on 2–4 year–old specimens from the same species [3], it can be asserted that growth causes considerable variations of certain hematic parameters. Other investigators verified that *Crocodylus palustris* mature specimens possess higher LDH concentration and lower ALP activity than the juvenile ones [18], in agreement with the changes here registered.

ALP decrease registered in present study, which were simultaneous to the advance of caymen growth, should necessarily be related to osseous development gradual decline, just as it happens in the rest of vertebrates [1, 4, 10]. This asseveration would be sustained by the significant negative correlation verified between weight evolution and ALP serum activity.

Winter fall of most studied enzymes maybe has its origin on the alimentary restriction conditioned by low temperatures, when metabolism decreases toward a saving phase [4, 7]. In other aquatic animals, as fishes, underfeeding generates energy reserves exhaustion (glucogen, lipids) and structural proteins consumption (muscles), causing growth detention and weight loss [11]. This circumstance causes decrease of serum ALT and LDH activities [12], just as it happened in the present study. In consonance, caymen reared at 18ºC registered lower weight gains than control specimens maintained at 22ºC [16]. Malnutrition states as consequence of winter

depletion from tissular reserves, would be frequent in crocodiles [17].

Confirming the diagnostic utility of enzymatic determinations on crocodiles, a lot of clinically "normal" *Alligator mississippiensis* specimens which had shared the same habitat with other caymen, which died after suffering neurological and muscular symptoms (intoxication?), revealed low ALP activity (31 IU/l), high levels from ALT (84 IU/l), AST (370 IU/l) and CHE (1342 IU/l), as well as very high CPK rates (42,500 IU/l) [17]. Last enzyme is liberated during nervous and/or muscular damages [1, 4, 10].

As conclusion, it can be enunciated that study gives a new reference interval for main enzymatic activities for clinical use, obtained on numerous *C. latirostris* and *C. yacare* sub–adults specimens, by conventional laboratory techniques, subject to appropriate quality controls. Physiological variations among species, sexes, ages and year seasons, are established. It is expected that obtained values can be useful for metabolic–nutritional control and illnesses diagnosis of caymen.

Acknowledgements. To Wiener Lab for supply the analytical kits used in the study. To Santiago Fioranelli and Gabriela Koza for their help in laboratory works. To M.S. Troia and E. Quirch de Troia for the revision of English grammar.

REFERENCES

1. **Angel G, Angel M**. 2000. *Interpretación Clínica del Laboratorio*, 6º ed., Panamericana, Bogotá, 664 p.
2. **Boló Bolaño E, Prado W, Moreno D**. 2000. Conservación y uso sustentable de yacaré overo (*Caiman latirostris*) y negro (*Caiman jacare*) en la provincia del Chaco. *Anales XV Reunión de Comunicaciones de la Asociación Herpetológica Argentina*, Bariloche, Argentina, p. 18.
3. **Canfield PJ**. 1985. Characterization of the blood cells of Australian crocodiles (*Crocodylus porosus* and *C. johnstoni*). *Zentralbl Vet Med C Anat Histol Embryol* 14: 269–288.
4. **Coppo JA**. 2001. *Fisiología Comparada del Medio Interno*, Ed. Dunken, Buenos Aires, 297 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* Nº 55, Anexo III: 1–15.
6. **Foggin CM**. 1987. Diseases and disease control on crocodile farms in Zimbabwe. In: *Wildlife Management: Crocodiles and Alligators* (Webb GJ, Manolis SC, Whitehead PJ, Ed.), Surrey Beatty, Chipping Norton, p. 351–362.
7. **Goldstein L**. 1997. *Fisiología Comparada*, 3º ed., Interamericana, México, 454 p.
8. **Gruss J, Waller T**. 1998. *Diagnóstico y Recomendaciones sobre la Administración de Recursos Silvestres en Argentina*, Ed. World Wildlife Foundation, Buenos Aires, 113 p.
9. **Jacobson E**. 1984. Immobilization, blood sampling, necropsy techniques and diseases of crocodilians: a review. *J Zoo Anim Med* 15: 38–45.
10. **Kaneko JJ**. 1989. *Clinical Biochemistry of Domestic Animals*, 4th ed., Academic Press, San Diego, 832 p.
11. **Machado CR, Garófalo MA, Roselino JE, Kettelhut IC, Migliorini RH**. 1988. Effects of starvation, refeeding, and insulin on energy–linked metabolic processes in catfish (*Rhamdia hilarii*) adapted to a carbohydrate–rich diet. *Gen Comp Endocrinol* 71: 429–437.
12. **Méndez G, Wieser W**. 1993. Metabolic responses to food deprivation and refeeding in juveniles of *Rutilus rutilus* (Teleostei: Cyprinidae). *Environ Biol Fishes* 36: 73–81.
13. **Millan JM, Janmaat A, Richardson KC, Chambers LK, Formiatti KR**. 1997. Reference ranges for biochemical and haematological values in farmed saltwater crocodile (*Crocodylus porosus*) yearlings. *Aust Vet J* 75: 814–817.
14. **Minucci PA, Waller T**. 1995. Los yacarés en Argentina. En: *La Conservación y el Manejo de Caimanes y Cocodrilos en América Latina* (Larriera A, Verdade L, Ed.), Ed. Fundación Banco Bica, Santa Fe, Argentina, 112 p.
15. **Moreno D**. 1998. Yacaré: Un recurso olvidado. *Rev Fund Vida Silv Arg* 62: 24–29.
16. **Piña C, Larriera A**. 2002. *Caiman latirostris* growth: the effect of a management technique on the supplied temperature. *Aquaculture* 211: 387–392.
17. **Schoeb TR, Heaton–Jones TG, Clemmons RM, Carbonneau DA, Woodward AR, Shelton D, Poppenga RH**. 2002. Clinical and necropsy findings associated with increased mortality among american alligators of Lake Griffin, Florida. *J Wildl Dis* 38: 320–337.
18. **Stacy BA, Whitaker N**. 2000. Hematology and blood biochemistry of captive mugger crocodiles (*Crocodylus palustris*). *J Zoo Wildl Med* 31: 339–347.
19. **Troiano JC, Althaus R**. 1994. Hallazgos hematológicos en *Caiman latirostris* (Crocodylia: Alligatoridae) en condiciones de cautiverio. *Memorias del IV Workshop sobre Conservación y Manejo del Yacaré Overo*, Santo Tomé (Santa Fe, Argentina), p. 12–24.
20. **Uhart M, Prado W, Beldoménico P, Rossetti C, Ferreyra Armas MC, Martínez A, Bardón JC, Avilés G, Karesh W**. 2001. Estudios sanitarios comparativos de yacarés (*Caiman latirostris* y *Caiman yacare*) silvestres y cautivos. *Boletín Técnico de la Fundación Vida Silvestre Argentina* Nº 55: 39–50.
21. **Waller T, Minucci PA**. 1993. Relevamiento de la distribución, hábitat y abundancia de los crocodilios de la Provincia de Corrientes, Argentina. *Anales I Reunión Regional del Grupo de Especialistas en Cocodrilos*, Santa Marta, Colombia, p. 341–385.
22. **Watson PA**. 1990. Effects of blasting on Nile crocodiles, *Crocodylus niloticus*. *Proceedings of the 10th Working Meeting of the Crocodile Specialist Group IUCN*, Gainsville, Florida, p. 240–252.