BLOOD AND URINE PHYSIOLOGICAL VALUES IN CAPTIVE BULLFROG, *Rana catesbeiana* (ANURA: RANIDAE)

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ABSTRACT: *Samples of healthy* Rana catesbeiana *(302 specimens, 9-21 months-old, 50% each sex) from the north-east of Argentina, were analyzed by spectrophotometry, electrophoresis, densitometry, refractometry and microscopy in order to obtain blood and urine reference values. Confidence intervals (p<0.05) for PCV (28.6-31.6%), RBC (0.40-0.44 T/L), MCV (686-732 fL), hemoglobin (6.41-7.20 g/dL), MCH (151-164 pg), MCHC (22.6-24.0%), WBC (18.7-22.3 G/L), neutrophils (58.4-63.4%), lymphocytes (23.9-29.8%), monocytes (2.1-3.8%), eosinophils (4.6-7.0%), basophils (2.9-4.1%), fibrinogen (0.59-0.99 g/dL), total protein (4.19-4.49 g/dL), albumin (1.49-1.67 g/dL), globulins (2.64-2.97 g/dL), creatinine (4.09-5.56 mg/L), urea (76.1-92.4 mg/L), uric acid (11.5-15.4 mg/L), triglycerides (0.34-0.52 g/L), total cholesterol (0.56-0.67 g/L), HDL-C (0.03-0.05 g/L), LDL-C (0.34-0.44 g/L), alpha lipoprotein (6.01-8.67%), beta lipoprotein (91.3-93.9%), glucose (0.45-0.54 g/L), Na (116-121 meq/L), K (3.42-3.81 meq/L), Cl (100-116 meq/L), Ca (7.98-8.61 mg/dL), P (8.31-9.36 mg/dL), Mg (2.26-2.55 mg/dL), Fe (105-178 ug/dL), ALP (144-170 IU/L), ALT (10.0-14.8 IU/L), AST (42.8-53.4 IU/L), GGT (7.8-10.6 IU/L), LDH (99-135 IU/L), CHE (151-185 IU/L), CPK (365-500 IU/L), bleeding time (289-393s), coagulation time (452-696s), prothrombin time (76-128s), urinary density (1.0061-1.0089), and urinary pH (6,38-6.96), were obtained. Some intervals were similar to those obtained in amphibians, birds or mammals, but others were very different. Physiological variations attributable to age, sex, season, and handling and feeding system, were registered on certain parameters. The usefulness of the parameters studied to evaluate sanitary, metabolic and nutritional state on captive* bullfrog *should be emphasized.*

Key words: *Rana catesbeiana*, blood values, coagulation tests, urine parameters

VALORES FISIOLÓGICOS EN SANGRE Y ORINA DE RANA TORO EN CAUTIVERIO, *Rana catesbeiana* (ANURA: RANIDAE)

RESUMEN: *Con el propósito de obtener valores sanguíneos y urinarios de referencia, 302 muestras de ejemplares sanos de* Rana catesbeiana *del nordeste argentino (9-21 meses de edad, 50% de cada sexo), fueron analizadas por espectrofotometría, electroforesis, densitometría, refractometría y microscopía. Fueron obtenidos intervalos de confianza (p<0,05) para hematocrito (28,6-31,6%), eritrocitos (0,40-0,44 T/L), VCM (686-732 fL), hemoglobina (6,41-7,20 g/dL), HCM (151-164 pg), CHCM (22,6-24,0%), leucocitos (18,7-22,3 G/L), neutrófilos (58,4-63,4%), linfocitos (23,9-29,8%), monocitos (2,1-3,8%), eosinófilos (4,6-7,0%), basófilos (2,9-4,1%), fibrinógeno (0,59-0,99 g/dL), proteínas totales (4,19-4,49 g/dL), albúmina (1,49-1,67 g/dL), globulinas (2,64-2,97 g/dL), creatinina (4,09-5,56 mg/L), urea (76,1-92,4 mg/L), ácido úrico (11,5-15,4 mg/L), triglicéridos (0,34-0,52 g/L), colesterol total (0,56-0,67 g/L), C-HDL (0,03-0,05 g/L), C-LDL (0,34-0,44 g/L), alfa lipoproteína (6,01-8,67%), beta lipoproteína (91,3-93,9%), glucosa (0,45-0,54 g/L), Na (116-121 meq/L), K (3,42-3,81 meq/L), Cl (100-116 meq/L), Ca (7,98-8,61 mg/dL), P (8,31-9,36 mg/dL), Mg (2,26-2,55 mg/dL), Fe (105-178 ug/dL), ALP (144-170 UI/L), ALT (10,0-14,8 UI/L), AST (42,8-53,4 UI/L), GGT (7,8-10,6 UI/L), LDH (99-135 UI/L), CHE (151-185 UI/L), CPK (365-500 UI/L), tiempo de sangría (289-393s), tiempo de coagulación (452-696s), tiempo de protrombina (76-128s), densidad urinaria (1,0061-1,0089) y pH urinario (6,38-6,96). Algunos intervalos fueron semejantes a los obtenidos en anfibios, aves o mamíferos, pero otros resultaron muy diferentes. Ciertos parámetros registraron variaciones fisiológicas atribuibles a edad, sexo, estación del año y sistema de manejo y alimentación. Se resalta la utilidad de los parámetros estudiados para evaluar estados sanitario, metabólico y nutricional de la rana toro en cautiverio.*

Palabras claves: *Rana catesbeiana*, valores sanguíneos, pruebas coagulativas, parámetros urinarios

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INTRODUCTION

Rana catesbeiana Shaw 1802 (bullfrog) has its origin in North America. Specimens present in Argentina come from genetic lines imported from Brazil, and they are adapted to tropical climate (1). There are more than 200 bullfrog hatcheries in Argentina which produce meat marketed at a high price (2). The meat is eatable and it is well-regarded because it has a scarce fat and cholesterol proportion (3). Bullfrog is characterized by its size; in captivity they can reach 300 g liveweight after 12 months. Since aging causes a decrease in the food conversion index, frogs which are to be sold later on are sacrificed when they are 6-7 months old, with approximately 170-180 g (4). *R. catesbeiana* is generally fed with balanced pellets which are similar to those elaborated for fish as its true nutritional requirements are still unknown (2).

The climate of northeastern Argentina is mainly warm and it favors hatcheries to respond the main market demand, that is, the continuity of production along the year (1). Indoor captivity is the system chosen to rear this animal as escapes would be dangerous to the ecosystem. *R. catesbeiana* is ecologically considered as an «undesirable guest», because when it settles in any lagoon, original aquatic fauna could rapidly become extinct due to its voracious appetite; cannibalism would not be unusual in this species (4). On the contrary, a natural diet based on autochthonous terrestrial anurous like *Bufo* sp. would be mainly compounded by coleopterons and hymenopterons (5).

Blood and urine composition would be influenced by peculiar physiological characteristics of the amphibian, such as metamorphosis, water and solutes skin exchange, capacity to support hemodilution and hemoconcentration, modification of urinary bladder water permeability, metabolic and enzymatic changes due to temperature, fast during winter lethargy, and others (6-9). Contrary to their close relatives (reptilians and birds) which are uricotelics, adult *R. catesbeiana* is ureotelic, although in tadpole stage it reveals an ammoniotelic pattern of nitrogen excretion (6). Frogs' blood is hyperosmotic in relation to the fresh water they live in, and urine is hyposmotic in relation to their blood (10). Corporal fluids pH varies according to body temperature, being acidified when temperature increases and *vice versa*; exchange of Na between cell and internal environment would also be altered by pH; changes of pH provokes numerous hematic and urinary modifications (10).

Except in the case of plasmatic electrolytes, texts of animal physiology reveal a manifest absence of hematic and urinary normal values from amphibians diagnostic parameters. Such parameters would be useful to evaluate health state in captive *R. catesbeiana*, which can suffer malnutrition, anemia, stress, transmissible diseases, intoxications, hemorrhagic dysfunctions, as well as inflammation and necrosis of liver, lungs, kidneys, spleen, muscles and other organs (4, 11).

The objective of this study was to obtain reference values and physiological variations from hematic and urinary diagnostic parameters in *R. catesbeiana*.

MATERIALS AND METHODS

Experimental subjects, feeding and handling

For a period of two years of studies, 302 healthy *R. catesbeiana* specimens were used. Two hundred and seventy of them were maintained on intensive systems, divided in 3 different frog farms in the north-east of Argentina. Samples from 90 frogs (9-21 months old, 50-350 g liveweight, 50% each sex), were taken in each breeding place. Thirty six per cent of the samples was taken during winter time, and 64% during the remaining seasons. No heating system during winter season was used in the hatcheries; all of them supplied food (45% protein balanced pellets, milled bovine lung, worms and fly larvas) at a ratio of 3-5% liveweight/day. The 32 remaining animals were reared on an extensive system (semi-captivity), in a closed lagoon where frogs selected exclusively "natural" food. They were adult 16-20 month-old animals from both sexes. Samples were taken during winter and all along the rest of seasons.

Sample Taking

Frogs were transported to the laboratory in thermal boxes which contained a NaCl 0.6% isotonic solution cooled with ice (2-3 ºC); this procedure causes desensitization and lethargy, facilitating the animal manipulation (4). Liveweight was obtained in an electronic balance Scientech-SL, with a 0.01 g accuracy. Samples were taken in the morning (7-8 AM), after a 24 h fasting period. Blood was obtained by intracardiac puncture, carried out with syringe and needle. The sample was a venose and arterial blood mixture, since frogs, with their anatomical characteristic, possess a unique ventricle (6). Some of the blood was treated with anticoagulant (EDTA, 0.34 mol/L), another was mixed with a sodium citrate

solution (130 mmol/L) and the last one was centrifuged (700g, 10 min) to obtain serum. Urine was obtained by cystocentesis.

Laboratory procedures

Being amphibians erythrocytes nucleated cells, erythrogram parameters were obtained applying avian techniques (12). There was a previous blood hemolysis and centrifugation to eliminate erythrocyte nuclei, and hemoglobin was later evaluated by photocolorimetry (Drabkin technique, using Wiener Lab reagents). Red blood cells (RBC) concentration was determined by means of Neubauer hemocytometer microscopic count using Biopur diluters, and the packed cell volume (PCV, hematocrit) was measured by capillary centrifugation (12,000 g, 5 min). White blood cells (WBC) concentration was obtained from stained smear count (Giemsa), considering corrections according to PCV value. Differential leukocyte count was carried out from stained smear (May Gründwald). Blood cells size was measured with an ocular micrometer. Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC), were obtained by conventional calculation.

Bleeding, coagulation and prothrombin time were evaluated respectively by Dukes, Lee-White and Quick methods (13). Fibrinogen was calculated by the difference between plasma and serum proteins (12), using an Erma-D refractometer. Urinalysis (density, pH, sediment, and chemical composition) was carried out by conventional laboratory techniques (10). Sodium and potassium were evaluated using Biopur reagents, in a Metrolab 305-D flame photometer. The separation of proteins (albumin and alpha, beta and gamma globulins, on cellulose acetate) and lipoproteins (alpha and beta, on agarose gel) was carried out by electrophoresis (13). Fractions were quantified in a Citocon CT-440 densitometer.

Serum biochemical parameters were measured in a Labora Mannheim 4010 UV-visible spectrophotometer, using Wiener, Boehringer and GT-Lab reagents, through regular laboratory methods: total protein (biuret), creatinine (alkaline picrate), urea (urease), uric acid (uricase), triglycerides (lipase peroxidase), total cholesterol (cholesterol oxidase), cholesterol linked to high density lipoprotein, HDL, and low density lipoprotein, LDL, (separation by precipitation), glucose (Trinder), chloride (mercuric tiocianate), calcium (cresolphtaleincomplexone), inorganic phosphorous (phosphomolybdate), magnesium (calmagite), iron (PBTS), and activities of alkaline phosphatase (ALP, phenylphosphate), alanine aminotransferase (ALT, oxoglutarate-NADH), aspartate aminotransferase (AST, aspartate-ketoglutarate), gammaglutamyl transferase (GGT, p-nitroanilide kinetic method), lactate dehydrogenase (LDH, dinitrophenylhydrazine), butyryl cholinesterase (CHE, kinetic with butyril-thiocholine) and creatine phosphokinase (CPK, creatine-ATP) (13).

Statistical analysis

The normality of the distribution of dependent variables (quantitative continuous) was assessed using the Wilk-Shapiro test (WS). Parametric descriptive statistics included measures of central tendency (arithmetic mean, $\bar{x}$), dispersion (standard deviation, SD) and ranges. Fiduciary probability was assessed by confidence intervals (CI±95%). Correlation coefficients were obtained by the Pearson procedure. Analysis of variance (ANOVA) was calculated by one way linear model, and mean comparison was made by Tukey test. For all inferences a 5% significance was specified, below which the equality null hypothesis was rejected. Calculations were all made using the *Statistix* software, 1996 version.

RESULTS

Values obtained from hemogram, coagulation tests, and some urinalysis parameters (Table 1), as well as from serum chemical values (Table 2), showed an approximately normal distribution, which allowed the use of parametric statistics. Confidence intervals were adjusted around arithmetic means, but individual ranges were wide. Correlation between age and weight was significant ($r = 0.82$, $p = 0.02$). Physiological variations due to age, sex, season, and feeding and housing systems, were also registered (Table 3).

Chemical tests on urine revealed that 7.6% of the studied amphibians had protein vestiges (30 mg/dl) and 4% showed bilirubin traces, which coexisted with small quantities of ketones. In all cases glucose was negative. Hemoglobin vestiges were verified in 57.6% of the samples. Urobilinogen was found in 100% of the studied amphibian urinary samples, with concentrations of 0.2 mg/dl (92% of animals) and 1 mg/dl (8% of animals). Scarce quantities of erythrocytes (57.6% of cases), leukocytes (15.3%), germs (53.8%) and granular cylinders (8%), were verified in the urinary sediment. No crystals were found in these amphibians' urine.

Table 1. Values obtained in bullfrog blood and urine (n = 302).
Tabla 1. Valores obtenidos en sangre y orina de rana toro (n = 302).

parameter	$\bar{x} \pm SD$	WS	CI±95%	range
PCV (%)	30.1 ± 5.4	0.936	28.6 - 31.6	25 - 39
RBC, concentration (T/L)	0.42 ± 0.07	0.952	0.40 - 0.44	0.31 - 0.59
RBC, length (um)	24.2 ± 1.9	0.942	23.7 - 24.8	20.5 - 27.7
RBC, breadth (um)	16.2 ± 1.3	0.939	15.8 - 16.6	13.2 - 19.5
MCV (fL)	709 ± 136	0.969	686 - 732	505 - 788
hemoglobin (g/dL)	6.80 ± 1.48	0.929	6.41 - 7.20	5.12 - 11.06
MCH (pg)	157 ± 22	0.966	151 - 164	121 - 197
MCHC (%)	23.3 ± 2.7	0.951	22.6 - 24.0	20.2 - 31.4
WBC (G/L)	20.5 ± 4.6	0.966	18.7 - 22.3	11.6 - 32.7
neutrophils, ratio (%)	60.9 ± 12.4	0.985	58.4 - 63.4	40.0 - 86.1
neutrophils, diameter (µm)	15.2 ± 2.1	0.945	14.6 - 15.7	11.3 - 20.5
lymphocytes, ratio (%)	26.8 ± 4.9	0.982	23.9 - 29.8	16.3 - 39.8
lymphocytes, diameter(µm)	13.6 ± 1.9	0.950	13.2 - 14.1	10.3 - 19.5
monocytes, ratio (%)	2.9 ± 1.1	0.942	2.1 - 3.8	1.0 - 5.0
monocytes, diameter (µm)	15.2 ± 2.1	0.949	14.7 - 15.8	10.2 - 20.5
eosinophils, ratio (%)	5.8 ± 1.6	0.935	4.6 - 7.0	2.0 - 11.9
eosinophils, diameter (µm)	16.2 ± 2.5	0.938	15.5 - 16.9	11.3 - 21.5
basophils, ratio (%)	3.5 ± 1.2	0.929	2.9 - 4.1	0 - 6.0
basophils, diameter (µm)	16.9 ± 2.8	0.931	16.1 - 17.7	11.3 - 21.5
bleeding time (s)	341 ± 67	0.956	289 - 393	240 - 490
coagulation time (s)	574 ± 98	0.973	452 - 696	360 - 788
prothrombin time (s)	102 ± 19	0.959	76 - 128	70 - 150
fibrinogen (g/dL)	0.79 ± 0.11	0.941	0.59 - 0.99	0.66 - 0.97
urinary density	1.0075± 0.0034	0.945	1.0061- 1.0089	1.0050- 1.0200
urinary pH	6.68 ± 0.71	0.939	6.38 - 6.96	5.00 - 8.50

$\bar{x}$: arithmetic mean, SD: standard deviation, WS: Wilk-Shapiro distributive normality test (chart coefficient: 0.947, α = 0.05), CI±95%: 95% confidence interval, PCV: packed cell volume, RBC: red blood cells, T/L: Tera/liter, um: micrometer, MCV: mean corpuscular volume, fL: femtoliter, MCH: mean corpuscular hemoglobin, pg: picogram, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cells, G/L: Giga/liter.

DISCUSSION

After food ingestion, changes in amphibian plasma composition would be registered (9). Other changes would also occur as consequence of circadian rhythm, caused by cortisol fluctuations (10). Both postprandial and circadian effects were excluded from the present study design due to previous fast and basal condition of samples, and also because blood extraction was carried out in uniform morning hours. Scarce regulation mechanisms and higher tolerance to hemodilution and hemoconcentration, would cause a great oscillation of blood values in frogs (6). This fact could explain the wide extent of ranges obtained in this trial. Correlation between age and weight was only moderately significant (r = 0.82, p = 0.02), probably because of the growth delay which takes place during the winter (4).

Erythrogram

In the present study, PCV values (30.1±5.4%) were higher than those reported on *R. catesbeiana*: 22±5% (14), but they matched with the reference interval published to *Rana tigrina*: 19.5-31.8% (15). Terrestrial toads *(Bufo sp.)* would register PCV values ranging from 36 to 44% (10). The *R. catesbeiana* hematocrit would not be very different from the values found on fowls: 25-35% (10) and 22-35% (16), but it would be lower than those reported on domestic mammals: 32-45% (17) and 37-47% (10).

Erythrocyte concentration would be ex-

Table 2. Values obtained in bullfrog serum (n = 302).
Tabla 2. Valores obtenidos en suero de rana toro (n = 302).

parameter	$\bar{x} \pm SD$	WS	CI±95%	range
total protein (g/dL)	4.34 ± 0.66	0.987	4.19 - 4.49	3.05 - 5.65
albumin (g/dL)	1.58 ± 0.33	0.954	1.49 - 1.67	1.02 - 2.67
alpha-1 globulin (g/dL)	0.22 ± 0.05	0.939	0.20 - 0.24	0.11 - 0.46
alpha-2 globulin (g/dL)	0.51 ± 0.09	0.964	0.48 - 0.54	0.30 - 0.65
beta globulin (g/dL)	0.72 ± 0.16	0.983	0.68 - 0.77	0.31 - 1.14
gamma globulin (g/dL)	1.35 ± 0.28	0.953	1.28 - 1.42	1.03 - 1.99
albumin/globulin ratio	0.54 ± 0.12	0.981	0.50 - 0.58	0.25 - 0.79
creatinine (mg/L)	4.83 ± 1.22	0.961	4.09 - 5.56	1.07 - 12.3
urea (mg/L)	84.2 ± 17.5	0.935	76.1 - 92.4	30.1 - 180
uric acid (mg/L)	13.4 ± 2.89	0.964	11.5 - 15.4	1.3 - 30.2
triglycerides (g/L)	0.43 ± 0.10	0.938	0.34 - 0.52	0.02 - 1.26
total cholesterol (g/L)	0.62 ± 0.14	0.927	0.56 - 0.67	0.30 - 1.18
HDL-C (g/L)	0.04 ± 0.01	0.923	0.03 - 0.05	0.01 - 0.10
LDL-C (g/L)	0.39 ± 0.09	0.949	0.34 - 0.44	0.18 - 0.83
alpha lipoprotein (%)	7.34 ± 1.85	0.921	6.01 - 8.67	2.00 - 24.6
beta lipoprotein (%)	92.65 ± 4.62	0.930	91.3 - 93.9	75.4 - 98.0
glucose (g/L)	0.50 ± 0.12	0.982	0.45 - 0.54	0.10 - 0.98
Na (meq/L)	118.6 ± 11.2	0.943	116 - 121	99 - 144
K (meq/L)	3.62 ± 0.71	0.974	3.42 - 3.81	1.92 - 5.84
Cl (meq/L)	108.6 ± 6.3	0.921	100 - 116	103 - 116
Ca (mg/dL)	8.31 ± 1.42	0.973	7.98 - 8.61	6.0 - 11.2
P (mg/dL)	8.83 ± 1.80	0.985	8.31 - 9.36	4.1 - 13.7
Mg (mg/dL)	2.41 ± 0.49	0.972	2.26 - 2.55	1.33 - 4.09
Fe (ug/dL)	142.1 ± 29.6	0.969	105 - 178	96 - 184
ALP (IU/L)	157 ± 32	0.959	144 - 170	73 - 248
ALT (IU/L)	12.4 ± 2.6	0.941	10.0 - 14.8	7 - 20
AST (IU/L)	48.1 ± 9.3	0.947	42.8 - 53.4	23 - 80
GGT (IU/L)	9.2 ± 1.6	0.932	7.8 - 10.6	5 - 20
LDH (IU/L)	117 ± 22	0.940	99 - 135	50 - 260
CHE (IU/L)	168 ± 32	0.975	151 - 185	45 - 274
CPK (IU/L)	432 ± 85	0.937	365 - 500	156 - 919

$\bar{x}$: arithmetic mean, SD: standard deviation, WS: Wilk-Shapiro distributive normality test (chart coefficient: 0.947, α = 0.05), CI±95%: 95% confidence interval, HDL-C: cholesterol linked to high density lipoprotein, LDL-C: cholesterol linked to low density lipoprotein, IU/L: International Units by liter, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gammaglutamyl transferase, LDH: lactate dehydrogenase, CHE: butyryl cholinesterase, CPK: creatine phosphokinase.

traordinarily variable in amphibians (6). RBC values found in these frogs (0.42±0.07 T/L) were considerably lower than those reported on fowls: 2.5-3 T/L (10), 3 T/L (17), 2.2-5.1 T/L (12), and 2.5-3.5 T/L (16), as well as those published on domestic mammals: 6.7-9.3 T/L (10), 7-14 T/L (17), 6-13 T/L (12), and 5-18 T/L (16). Erythrocyte dimensions in this study (length x breadth = 24.2x16.2 µm) were approximately similar to those found on *R. catesbeiana*: 24x14 µm (18), but they were smaller than those reported on birds: 12x7 µm (16,17), and domestic mammals: 3.2-9.6 µm (12), 3.2-7 µm (16), and 3.9-7.2 µm (17). Our MCV (709±136 fL) was higher than those published on fowls: 105-115 fL (10), 115-125 fL (17), and 90-140 fL (16), as well as those reported on domestic mammals: 45-65 fL (10), 19-69 fL (17), 19-70 fL (12), and 16-77 fL (16).

Hemoglobin concentration of the studied frogs (6.80±1.48 g/dL) was higher than the one found on *R. catesbeiana*: 4.7±0.9 g/dL (14), and *R. tigrina*: 3.87-6.22 g/dL (15). This respiratory pigment would be higher on toads: 10-13 g/dL (10), and birds: 8-12 g/dL (17), 7-13 g/dL (16), and 7.2-9.6 g/dL (10), as well as on domestic mammals: 12-16 g/dL (10), 11-14 g/dL (17), 11-15 g/dL (12), and 8-19 g/dL (16,19). Since a frog MCV would be higher compared to the one found on other species, the MCH index (157±22 pg) was also higher than the values reported on birds: 29-33 pg (10), 25-27 pg (17), 33-47 pg (16), and mammals: 16-25 pg (10), 13-31 pg (17), 7-23 pg (12), and 5.2-24.5 pg (16). Our MCHC (23.3±2.7%) was simi-

Table 3. Physiological variations of some studied parameters (x).
Tabla 3. Variaciones fisiológicas de algunos parámetros estudiados (x).

parameter	season		sex		age		handling	
	cold	warm	male	female	young	adult	captiv.	semic.
glucose (g/L)	0.39[a]	0.61[b]	0,51	0.48	0.66[a]	0.41[b]	0.53	0.56
urea (mg/L)	90.1[a]	79.5[b]	87.1	82.6	65.0[a]	113[b]	80.1[a]	105[b]
creatinine (mg/L)	4.87	4.79	5.06	4.64	3.12[a]	7.71[b]	5.25	5.66
uric acid (mg/L)	14.4	12.6	14.0	13.1	19.6[a]	5.3[b]	12.7	14.8
Na (meq/L)	117	120	116	121	129	111	128	130
K (meq/L)	3.2[a]	4.1[b]	3.6	3.7	2.7[a]	4.2[b]	3.8	3.9
Ca (mg/dL)	7.63[a]	9.22[b]	8.42	8.27	9.70[a]	8.02[b]	8.73	9.25
P (mg/dL)	7.83[a]	9.65[b]	8.69	8.94	10.9[a]	5.83[b]	10.4	9.75
Mg (mg/dL)	1.92[a]	3.03[b]	2.54	2.29	2.32	2.70	2.51	2.68
PCV (%)	27.2[a]	32.9[b]	30.0	30.3	26.4[a]	37.2[b]	33.8	34.5
RBC (T/L)	0.38[a]	0.46[b]	0.41	0.43	0.35[a]	0.49[b]	0.43[a]	0.45[b]
MCV (fL)	707	711	698	719	723	745	769	751
hemoglobin (g/dL)	5.92[a]	7.48[b]	6.78	6.82	5.90[a]	7.73[b]	7.32	7.50
MCH (pg)	154	159	155	160	174[a]	148[b]	159	162
MCHC (%)	22	23	23	24	23	22	23	22
WBC (G/L)	19.8[a]	21.3[b]	21.2	19.4	26.4[a]	13.1[b]	20.3	21.4
neutrophils (%)	60.2	61.5	62.8	60.7	53.7[a]	73.4[b]	62.4	58.7
lymphocytes (%)	24.7	29.1	25.0	27.2	40.0[a]	20.5[b]	29.1	28.0
monocytes (%)	2.8	3.1	3.1	2.7	4.2	2.1	2.3	3.5
eosinophils (%)	5.7	6.0	6.5	4.8	6.0	5.3	6.5	6.7
basophils (%)	3.1	3.8	2.6	4.6	3.6	2.9	3.8	3.6
ALP (IU/L)	171[a]	144[b]	168[a]	146[b]	196[a]	102[b]	151[a]	135[b]
ALT (IU/L)	14.2	11.4	11.3	12.5	12.1	9.3	12.0	10.4
AST (IU/L)	58.6[a]	39.3[b]	46.7	48.9	59.2	39.8	47.5[a]	40.8[b]
GGT (IU/L)	8.4	11.1	8.8	10.1	7.4	8.1	9.1	7.4
LDH (IU/L)	138[a]	94[b]	132	104	138	96	104	95
CHE (IU/L)	177[a]	156[b]	173	165	126[a]	226[b]	166	182
CPK (IU/L)	447	419	498	405	280[a]	572[b]	474	427
triglycerides (g/L)	0.25[a]	0.59[b]	0.40	0.45	0.62[a]	0.24[b]	0.51	0.57
tot. cholesterol (g/L)	0.59[a]	0.66[b]	0.65	0.60	0.76[a]	0.54[b]	0.65	0.71
HDL-C (g/L)	0.04	0.04	0.05	0.04	0.06	0.03	0.04	0.05
LDL-C (g/L)	0.37	0.42	0.41	0.36	0.54[a]	0.32[b]	0.45	0.46
alpha lipoprot. (%)	7.1	7.5	7.2	7.4	9.2[a]	5.4[b]	9.6	8.7
beta lipoprot. (%)	92.8	92.5	91.8	94.0	90.8[a]	94.6[b]	90.3	91.5
total protein (g/dL)	3.93[a]	4.71[b]	4.41	4.32	3.81[a]	4.90[b]	4.41[a]	4.84[b]
albumin (g/dL)	1.30[a]	1.78[b]	1.63	1.49	1.38[a]	1.80[b]	1.52[a]	1.80[b]
alpha-1 glob. (g/dL)	0.21	0.24	0.19	0.23	0.15	0.14	0.24	0.25
alpha-2 glob. (g/dL)	0.49	0.52	0.52	0.48	0.45	0.57	0.52	0.57
beta globulin (g/dL)	0.69	0.75	0.69	0.74	0.65[a]	0.89[b]	0.73	0.79
gamma glob. (g/dL)	1.32[a]	1.75[b]	1.39	1.33	1.17[a]	1.52[b]	1.40	1.43
albumin/glob. ratio	0.52	0.55	0.56	0.54	0.57	0.58	0.53	0.59

$\bar{x}$: arithmetic mean, captiv.: captivity, semic.: lagoon, PCV: packed cell volume, RBC: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cells, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gammaglutamyl transferase, LDH: lactate dehydrogenase, CHE: butyryl cholinesterase, CPK: creatine phosphokinase, tot.: total, lipoprot.: lipoprotein, glob.: globulin. In each line, different letters indicate significant differences ($p < 0.05$).

lar to those published on fowls: 21-23% (17) but lower than those found on domestic mammals: 32-37% (10), 27-39% (17), 31-35% (12), and 30-37% (16).

Nutritional anemias would be common in amphibian. Coccidia, as *Babesiosoma stableri*, would be located inside erythrocytes; *Lankesterella minima* would also parasite tadpoles and adult frog RBC (11). Hematocrit would decrease in anemias, and it would increase in dehydration and postprandial stage (10). The latter would be due to spleen RBC release (9). Hematocrit and hemoglobin would diminish as a consequence of alimentary deficiencies and prolonged fast (15). Erythrocytes indicators in the nutritional state panel show a decrease owing to insufficient protein, vita-

mins B_{12}, E, niacin and folic acid intake (17). Appropriate erythropoiesis would require a continuous and balanced affluence of minerals such as Fe, Cu, Co and Se; nutritional lacks would also cause hematocrit and hemoglobin decrease (16).

Leukogram

The WBC concentration found on *R. catesbeiana* by others: 5.2±2.9 G/l (14) was lower than the one obtained in the present trial (20.5±4.6 G/L), presumably because of a depression caused by the anesthetic used in sample taking. The WBC average found on these frogs coincides with the reference intervals reported on birds: 13-22 G/L (10) and 18-30 G/L (17), but it is higher than those published on domestic mammals: 6-12 G/L (10), 5-20 G/L (17), and 7.6-16 G/L (12).

Neutrophils ratio (60.9±12.4%) was higher than those reported on avian heterophils: 23-28% (10) and 27% (17), as well as those reported on ruminant neutrophils: 33-39% (10), 25-30% (17), and 10-50% (12,16). However, this ratio was similar to those published on carnivorous and monogastric herbivores: 58-64% (10), 60-75% (12,17), and 60-77% (16).

The percentage of lymphocytes (26.8±4.9%) was lower than those obtained on birds: 58-65% (10) and 59% (17), but it was similar to those published on carnivorous: 25-37% (10), 20-25% (17), and 12-30% (12,16). On the other hand, our ratio was lower than those found on ruminant: 55-61% (10), 55-65% (17), 48-75% (12), and 50-70% (16).

The monocyte proportion found in bullfrog (2.9±1.1%) matched with the reference interval admitted for the majority of domestic mammals and birds: 2-8% (17), 1-10% (12), 0-10% (16), and 2-6% (10). The quantity of eosinophils (5.8±1.6% on *R. catesbeiana*), was also similar to the one reported on domestic mammals: 1-6% (10), 2-12% (17), 1-15% (12), and 0-20% (16), but it was higher than the one obtained on birds: 2% (17) and 2-3% (10). Frogs would have basophils (3.5±1.2%) in a slightly higher proportion than those reported on domestic mammals and birds, whose values are 0-1% and 1-2% respectively (10,17).

The mean diameter of neutrophils on the frogs of the present study (15.2 µm) was lower than the one reported on *R. catesbeiana*: 25 µm (18), but it was higher than those published for domestic mammals: 10-12 µm, and birds (heterophils): 9.6 µm (17). The bullfrog lymphocyte size (13.6 µm) was similar to those found on the same frog species by other authors, from 8 to 18 µm (18), but it was lower than those published on birds: 18.2 µm, and mammals: 19-25 µm (17). The bullfrog monocyte diameter (15.2 µm) was slightly bigger than those reported on hens: 12.5 µm, and mammals: 12-14 µm (17). On *R. catesbeiana* eosinophils, other authors (18) report a diameter (24 µm) that is higher than the value found in this study (16.2 µm). Eosinophils would measure 9.7 µm on birds and 10-14 µm on domestic mammals (17). Basophils diameter found in bullfrog (16.9 µm) would be slightly higher than those published for birds: 10.3 µm, and mammals: 7-15 µm (17).

Leukogram is useful to evaluate infection, inflammation, stress, neoplasias and other dysfunctions (10). Amphibian leukocytes may possess properties different to those of mammals, because temperature would greatly affect the cellular inflammatory response (20). In *R. catesbeiana* tadpoles, lymphocytes and monocytes, but not granulocytes, would participate in the inflammatory focus development (21).

Coagulogram

No previous values reported for hemostatic tests on *R. catesbeiana* were found. The obtained bleeding time (341 s = 5.7 min) was similar to those published on human beings and domestic mammals: 2-5 min (10,12,17). The whole blood clotting time (574 s = 9.6 min) was neither different to those reported on mammals: 4-15 min (12) and 1-11 min (17), as well as on human beings: 6-9 min (10), although it would be shorter on birds, from 0.5 to 2 min (17).

Bullfrog prothrombin time (102 s) was markedly longer than those admitted as normal in human beings: 11-15 s (10), carnivorous: 12-15 s (19) and 6-7 s (16), ruminants: 20-30 s (19), and mammals in general: 12 s (10) and 10-28 s (17). Concentration of fibrinogen found in this trial (0.79±0.11 g/dL) was close to normal values reported on cows: 0.60-0.70 g/dL (10,16,17), but it was slightly higher than those published on fowls: 0.1-0.4 g/dL (16) and monogastric mammals: 0.1-0.5 g/dL (19) and 0.22 to 0.39 g/dL (10).

The fundamental triad of hemostasis exploratory tests in human beings and domestic animals is constituted by bleeding, coagulation and prothrombin times, which evaluate platelet function, intrinsic pathway, and extrinsic pathway respectively (10,12). In coagu-

lative anomalies is also important to determine the plasma fibrinogen concentration, to discard eventual hypo-, dis-, and a-fibrinogenemias provoked by hemorrhagic diathesis, hepatopathies, and malnutrition (13,19). Amphibian thrombocytes would provide the necessary factors to form thromboplastin, which would transform fibrinogen into fibrin (7).

The knowledge of coagulogram values from *R. catesbeiana* could contribute to clarify coagulopathies caused by inadequate diet, intestinal malabsorption, intoxications or metabolic disturbances (stress, cholestasis, fatty liver, myelodystrophies). Hypovitaminosis K causes cutaneous hemorrhages as prothrombin time increases. Perhaps the most important bullfrog coagulopathy is the red-leg syndrome; it is a septicemia that causes skin and skeletal muscle hemorrhages, cutaneous ulcers, inflammation and necrosis of liver, spleen, and other celomic organs, emaciation, and death. Diverse intoxications and hepatopathies can lead to a deficit of fibrinogen and/or other clotting factors (4,11).

Urinalysis

Urinary density obtained on bullfrog (1.0075±0.0034) was lower than those reported on mammals, from 1.015-1.045 (12) and 1.010-1.050 (19), as well as on birds, from 1.009 to 1.033 (12). The extremely low density obtained confirms that the urine of this frog is significantly hyposmotic. While the internal environment of mammals (endothermals) has an osmolarity equivalent to 0.8-0.9% saline solutions, in amphibians (ectothermals) it coincides with 0.6-0.7% saline solutions (10).

Water volumes equivalent to 30-50% of body weight can be stored in terrestrial amphibian urinary bladder, which is capable of absorbing water and salt against gradient, and cause urine hyposmolarity. Recent studies demonstrate that urinary vesical wall has the ability to regulate its water permeability. Protection against water loss is mainly based on the oliguria: urine will concentrate until it is isosmotic in relation to plasma. No amphibian can produce urine which could be hyperosmotic in relation to blood (22). Urine concentration mechanisms based on solutes resorption (until they are hypertonic to plasma), are characteristic of mammals, not amphibian (10).

Urinary pH registered in the present study was almost neuter (6.68±0.71). It is acid (up to 5) on carnivores, and alkaline on herbivores, up to 8.4 (12,19). Urinary pH would be from 5-8 on birds, diminishing up to 4.7 in aquatic species when they are submerged (12). Glucose tubular resorption would be total in this species, because its presence in urine was not verified in any of the cases; glucosuria is abnormal in all domestic animals (12,17). Urobilinogen found in the urine of the studied frogs would be normal, because it is the hemoglobin metabolism terminal product; it is habitually present in urine of both carnivorous and herbivores species (10).

On birds' urine, but not in those of mammals, it would be normal to discover vestiges of blood, bilirubin, protein and ketones (12), as it found in these frogs. Germs and cylinders presence is abnormal on mammals' urine, but the existence of epithelial (genital and urinary) cells is usual, as well as some leukocytes, such as those found in frogs. The presence of abundant phosphate, carbonate and urate crystals reported respectively in carnivores, herbivores and birds (12), contrasts with the absence of crystals in the urine of these frogs. Physicochemical characteristics verified on *R. catesbeiana* urine (low density, pH nearly neutral, absence of crystals) are in correspondence with those from species whose habitat facilitates the residues excretion without necessity of preserving great water quantities (10).

Proteinogram

Total protein frog values (4.34±0.66 g/dL) were quite different to the habitual values on mammals: 6.5-7.5 g/dL (17), 5.8-7.8 g/dL (12), 5.2-8.9 g/dL (19), 6-8.5 g/dL (16), and 6-8 g/dL (10). On the other hand, they were similar to those reported on terrestrial amphibians as *Bufo sp.*: 3.6-6 g/dL (10) and birds: 2-5.5 g/dL (12), 4-5.5 g/dL (16), and 3.8-4.5 g/dL (10). In the present study, bullfrog plasma albumin (1.58±0.33 g/dL) was similar to those published on toads: 1-1.9 g/dL, and birds: 1.6-2 g/dL (10), but it was lower than those reported on mammals: 3-3.9 g/dL (10), 2.8-3.2 g/dL (17), and 2.6-4 g/dL (12).

Alpha-1 globulin mean value (0.22±0.05 g/dL in these frogs) was similar to those found on toads: 0.3-0.4 g/dL, fowls: 0.1-0.2 g/dL, and domestic mammals: 0.2-0.4 g/dL (10). On mammals, unified values for alpha-1 and alpha-2 globulins would be 0.9-1.3 g/dL (17) and 0.42-0.63 g/dL (12). On the studied frogs, the value of alpha-2 globulin (0.51±0.09 g/dL) did not differ much from those published on toads: 0.4-0.5 g/dL, fowls: 0.3-0.4 g/dL, and mammals: 0.3-0.7 g/dL (10). Bullfrog beta globulin (0.72±0.16 g/dL) was smaller than those re-

ported on toads: 0.9-1.3 g/dL (10) and mammals: 0.8-1.1 g/dL (19) and 0.9-1.8 g/dL (10), but it was higher than those found on fowls: 0.5-0.7 g/dL (10).

In the present study, frogs gamma globulin values (1.35±0.28 g/dL) resulted similar to those found on toads: 1.2-1.8 g/dL, and fowls: 0.7-1.4 g/dL (10), but they were lower than those reported on mammals: 1.4-2.2 g/dL (17), and 0.9-2.7 g/dL (10). On these frogs, albumin / globulin ratio (0.54±0.12) was similar to that published on toads: 0.3-0.5 (10) and ruminants: 0.42-0.76 (19), but it was lower than those reported on fowls: 0.8-1.1 and carnivorous: 1.4 (10), as well as those published on horses: 0.62-1.46 (19).

On mammals, all albumins and half of globulins that circulate in blood, are synthesized in the liver; on amphibians, this function would be carried out by the hepatopancreas (6). Plasma proteins intervene in acid-base balance, immunity, coagulation, colloid-osmotic pressure, and blood viscosity; they also transport hormones, vitamins, lipids, bilirubin, calcium, zinc, iron and copper (17,19). Albumins are excellent indicators of protein biosynthesis; they also operate as nutritional reserve of amino acids, which would be habitually exchanged between plasma and tissues, mainly in skeletal muscles (10). Proteinogram is of clinical interest because it facilitates the diagnosis towards alterations such as alimentary lacks, malabsorption, hepatopathies, inflammations, and renal, coagulative, and immunologic dysfunctions (10,12,13).

Non proteic nitrogen (NPN)

Creatinine is the muscular creatine phosphate metabolic residue. Its value on the studied amphibians (4.83±1.22 mg/L) was similar to those found on toads: 3-6 mg/L (10), but it was lower than those published on birds: 5-15 mg/L (12), and mammals: 5-27 mg/L (19), 10-20 mg/L (12), and 9-19 mg/L (10).

Urea, the final product of protein metabolism, had a plasma value of 84 mg/L (0.08 g/L) on the trial frogs. This concentration was lower than those reported on mammals: 0.10-0.30 g/L (19), 0.20-0.44 g/L (10), 0.28-0.34 g/L (17), and 0.35-0.45 g/L (12). Uric acid (10,17) is the excretion residue of nucleic acids (mammals), and proteins (birds). Its value on bullfrog (13.4±2.89 mg/L) was similar to those found on toads: 9-16 mg/L (10) and domestic mammals: 0-20 mg/L (19), and 5-13 mg/L (10), but it was lower than those reported on fowls: 49-67 mg/L (10) and other birds: 25-140 mg/L (12).

These results confirm that *R. catesbeiana* is an ureotelic rather than uricotelic amphibian. Contrary to their uricotelic relatives (reptiles and birds), mature amphibian, as well as mammals, would excrete NPN in the form of urea, although in tadpole stage they would excrete ammonia (7). Exceptionally, some frogs (*Phyllomedusa sauvagii* and *Chiromantis xerampelina*) would excrete NPN in urate form, and some toads (*Xenopus laevis*) would be ureotelic during their permanency on earth, but they would become ammoniotelic when they are in water (6).

In spite of their ureotelic pattern, amphibians would retain urea to regulate their osmotic pressure. Environment salinity increase would cause urea retention because it increases the urea hepatic synthesis and decreases the urea renal excretion. This fact could be proved in *Rana cancrivora* specimens exposed to fresh water versus sea water. They registered differences in plasma osmolarity (290 versus 830 mOsm/l), urea (40 versus 350 mMol/l), sodium (125 versus 250 mEq/l) and urine flow (100 versus 1%) respectively (6). This clearly indicates that frogs utilize urea to maintain their hyperosmolarity with the environment (22).

NPN earns importance in diagnosis of renal failure, metabolic alterations and nutritional disturbances (10,13). In hatcheries, several infections, intoxications, and parasitosis (*myxosporea*) affect frogs kidneys; in the same sense, certain metabolic illnesses cause renal obstruction with NPN retention (4).

Lipidogram

Triglycerides level on the frogs of the present trial (0.43±0.10 g/L) was similar to those found on toads (0.31-0.73 g/L), and it was not so different from those reported on mammals: 0.26-0.95 g/L (10). On birds, triglyceridemia would be higher, reaching 2.2 g/L (17). Bullfrog total cholesterol (0.62±0.14 g/L) was higher than the one found on pigs: 0.36-0.54 g/L (19), but it was lower than those published on toads: 0.91-1.83 g/L (10), herbivore mammals: 0.77-1.73 g/L (17), carnivorous: 1.35-2.70 g/L (19), and birds: 1-2 g/L (12), 1 g/L (17), and 0.90-1.30 g/L (10).

The HDL-C value found on these frogs (0.04±0.01 g/L) was lower than those published (10) on human beings (0.4-0.6 g/L), horses (0.5-0.7 g/L) and dogs (0.8-1.2 g/L).

Frogs LDL-C concentration (0.39±0.09 g/L) was not quite different from those reported on dogs (0.2-0.6 g/L) and horses (0.2-0.4 g/L), but it was lower than those published on human beings: 0.9-1.6 g/L (10). In this study, alpha lipoprotein proportion (7.34%) was markedly lower than those published on fowls (67-75%), dogs (84-90%), cows (87-90%) and human beings (32-44%), and beta lipoprotein ratio (92.65%) was significantly higher than those found on dogs (10-16%), ruminants (10-13%), fowls (16-22%, with 8-12% of pre-beta lipoprotein) and human beings (42-55%, with 8-16% of pre-beta lipoprotein) (10).

Lipoprotein metabolism would reveal similar characteristics among different animal species, but it would not be exactly the same in all of them. Canine, feline, equine, ruminant and some rodents would have «HDL pattern», characterized by plasma alpha lipoprotein predominance. When these animals are fed on fatty diets, cholesterol is linked by HDL rather than LDL, avoiding noxious effects due to protective action attributable to HDL. Human beings, pigs, rabbits, marmots, and several monkey species, would respond to the «LDL pattern», because when they consume fat, they increase their beta lipoprotein and they are exposed to a major atherogenic risk (10,23). Bearing in mind that C-LDL level was higher than C-HDL level, and that alpha lipoprotein ratio was lower than beta lipoprotein ratio, frogs would join in the «LDL pattern» rather than the «HDL pattern». Similarly, other researchers found ratios of LDL higher than HDL in *Rana catesbeiana* plasma, although they also found vestiges of low density lipoprotein (VLDL), which was not detected in the present study (24).

Lipidogram values may vary due to age, heredity, food type, and diverse illnesses, such as hepatic and renal failure, malabsorption, stress, hypothyroidism, and infections. Cholesterol would rise in the initial phase of starvation (due to high fat mobilization), but in case of prolonged fast its plasma concentration tends to decrease (10,12,13,19).

Glucose

Glucose concentration on the studied frogs (0.50±0.12 g/L) was similar to the one reported on toads: 0.55-0.61 g/L (10) and ruminants: 0.45-0.70 g/L (12), but it was lower than those published on birds: 2.2-2.9 g/L (10), 1.3-2.6 g/L (17), and 1.9-4.5 g/L (12). Glucemia would also be higher in monogastric mammals: 0.83-0.91 g/L (10), 0.65-1.50 g/L (19), and 0.6-1.2 g/L (12).

In amphibians, glucemia would decrease during the stage previous to winter lethargy, with an increase of hepatic glycogen; hypoglucemia would cause hypothermia. Insulin would decrease glucemia and temperature in *R. catesbeiana*; on the contrary, high temperature would increase the O_2 consumption and it would cause hyperglucemia (25). Plasma glucose would be regulated through insulin, glucagon, adrenaline, cortisol, and thyroid hormones (6,7,22). Physiologically, glucemia might vary by effects of age and physical exercise; pathologically it would alter in malnutrition, stress, and endocrine and hepatic failures (10,12,19).

Ionogram

Sodium level in the studied frogs (118.6±11.2 meq/L) was similar to the levels reported on *R. catesbeiana*: 108±5 meq/L (14), and other frogs of fresh water: 109 meq/L (22) and 92-125 meq/L (7). Natremia would be slightly higher on toads: 128-139 meq/L (10), birds: 131-157 meq/L (12) and 130-146 meq/L (10), and domestic mammals: 132-156 meq/L (19), 110-155 meq/L (12), and 132-146 meq/L (10).

Plasma potassium concentration would be 2.4-6.7 meq/L (12,19) and 3.3-5.1 meq/L (10) on mammals; 2.5-4.5 meq/L (12) and 5.1-6.4 meq/L (10) on birds; 3.7-6.2 meq/L on toads (10), and 2.6 meq/L (22), 3 meq/L (7) and 2.7±0.71 meq/L (14) on frogs. The kalemia reported on amphibians was approximately similar to that found in this trial on *R. catesbeiana* (3.62±0.71 meq/L).

Chloride value in the studied frogs (108.6±6.3 meq/L) was slightly higher than those reported on frogs: 70-98 meq/L (7) and 77±6 meq/L (14), and toads: 85-96 meq/L (10). However, it was similar to the values published on mammals: 94-123 meq/L (19), 88-118 meq/L (12), and 93-112 meq/L (10).

There were no great differences registered between calcium concentration on studied frogs (8.31±1.42 mg/dL) and plasma calcium levels reported on the same species: 8.05±0.88 mg/dL (14), other frogs: 8.4 mg/dL (22), and 9.2 mg/dL (7), toads and fowls: 7.8-9.6 mg/dL and 9.3-10 mg/dL respectively (10), and mammals: 8-12 mg/dL (12), 6.2-13.6 mg/dL (19), and 8.4-11.5 mg/dL (10).

The value of inorganic phosphorous obtained in the present study (8.83±1.8 mg/dL) was higher than the one reported (14) on *R. catesbeiana* (3.3±0.7 mg/dL). Nevertheless, it

was similar to those obtained on toads and birds: 6.3-8.2 mg/dL and 6.2-8.7 mg/dL respectively (10). Amphibians phosphatemia would be higher to those published on mammals: 3-6 mg/dL (17), 3-8 mg/dL (12), 2.6-6.9 mg/dL (19), and 3-5.2 mg/dL (10).

In these frogs, plasma magnesium value (2.41±0.49 mg/dL) was similar to those found on bullfrog: 2.05±0.35 mg/dL (14), other fresh water frogs: 3.1 mg/dL (22), and 3.9 mg/dL (7), toads and birds: 2.3-4.2 mg/dL and 2-3 mg/dL respectively (10), as well as domestic mammals: 2.5-3 mg/dL (17), 1.8-3.7 mg/dL (19), 1.8-4 mg/dL (12), and 1-3 mg/dL (10). The iron level on the studied frogs (142.1±29.6 µg/dL) coincided with the reference interval reported on toads: 83-145 µg/dL (10), and domestic mammals: 100-180 µg/dL (17), 57-222 µg/dL (19), 86-193 µg/dL (16), and 93-165 µg/dL (10).

In frogs, electrolytes and water enter the organism through skin and digestive tract, being eliminated by skin, urine and feces; amphibians skin could check the osmolarity of the surrounding liquid (22). Fresh water frogs are hyperosmotic in its environment, that is the reason why they tend to incorporate water by the skin and decrease their corporal saline concentration (6). In these animals, high internal osmolarity (210-290 mOsm/L) and low external osmolarity (50 mOsm/L), could cause overhydration (the entry of water by osmotic gradient) and loss of electrolytes (diffusion by concentration gradient). Homeostasis is achieved with abundant hypotonic urine and an increase in electrolytes tubular resorption and salt cutaneous absorption (7).

Diverse illnesses can alter electrolytic homeostasis when disturbing the feedback of internal environment regulatory hormones (aldosterone, parathormone, calcitonin, vasopressin, thyroid, natriuretic factor), thus causing metabolic disturbances (10,12,13,19). Mineral nutritional deficiencies are frequent in frog hatcheries, especially related to calcium lack, which provokes osseous malformations (4).

Enzymogram

On frogs of the present study, ALP mean activity (157 IU/L) was considerably lower than that reported on birds: 2100-3200 IU/L (10), but it was similar to those published on pigs and dogs: 118-395 and 20-156 IU/L respectively (19), as well as to those found on human beings and domestic mammals: 95-185 and 90-230 IU/L respectively (10). Nonetheless, frog ALT (12.4 IU/L) was alike those from remaining compared species, such as human beings: 3-14 IU/L (10) and domestic mammals: 8-27 IU/L (17), 3-102 IU/L (19), and 6-13 IU/L (10).

AST activity (48.1 IU/L in studied frogs) was similar to that published on ruminants: 36-45 IU/L (10), although it was considerably lower than those reported on fowls: 270 IU/L (17) and 100-350 IU/L (12), and horses: 165 IU/L (17) and 152-225 IU/L (10). Human beings and canines would register lower AST activities: 4-19 IU/L and 8-15 IU/L respectively (10).

GGT enzymatic concentration on these frogs (9.2 IU/L) was not different from those reported on human beings: 9-36 IU/L (10) and domestic mammals: 1.2-13.4 IU/L (19) and 5-21 IU/L (10). Frog LDH (117 IU/L) was lower than those published on cows: 692-1445 IU/L (19), but it matched with the reference interval on birds: 75-650 IU/L (12), canines: 45-233 IU/L (19), domestic mammals and human beings: 72-153 and 62-148 IU/L respectively (10). In 11 anesthetized *R. catesbeiana* specimens, values of LDH lower than those obtained in this study (33±20 IU/L), were reported (14). The effect of anesthesia or the employment of another technique for the enzymatic assay could be the cause of such difference.

Frog CHE activity (168 IU/L) was markedly lower than in human beings: 3400-6800 IU/L (10). Higher values of this enzyme were also reported on horses (2000-3100 IU/L), and pigs (400 IU/L), although on goats and cows (110 and 70 IU/L respectively) they were lower (19). At the same time, frog CPK (432 IU/L) was higher than those published on remaining compared species, such as birds: 100-200 IU/L, and domestic mammals: 2-28 IU/L (19) and 39-85 IU/L (10).

Chronic hepatic disorders result in increased plasma ALP in most animals. During normal bone growth in young animals, a large amount of ALP is in plasma; osteopathies also results in increased plasma ALP. Recently, GGT has been found to be liver specific and is used as an indicator of hepatobiliary disease. Increased plasma AST is associated with cell necrosis of the liver and skeletal or cardiac muscle, starvation and lack of vitamin E. ALT is well established as a marker of acute hepatic damage. Injury to skeletal and cardiac muscle results in considerable increase in plasma CPK. Brain also contains great amounts of the latter. LDH is released after cellular damage to the liver, lung, muscle,

heart and kidney tissue. CHE is originated in liver, pancreas, intestinal mucosa and brain; decrease in CHE has been reported in liver failure, muscular dystrophy, chronic renal disease and organophosphate insecticide intoxication (10,12,13,19).

Physiological variations registered in present study would be attributable to combined actions of ontogeny (growing) (10), climate (winter lethargy) (8), hormonal effects (16), and handling (housing and feeding) systems (9).

The obtained data could be useful to optimize the diagnosis of sanitary, metabolic and nutritional dysfunctions in *R. catesbeiana*. It could also cooperate with the search of real nutritional requirement of this amphibian in captivity. Such knowledge may lead to an improvement in frog meat production, thus a promising future. Frog meat world consumption ranges between 30,000 and 50,000 tn/year, with an existing market for skin (leather crafts), intestine (esthetic surgery thread), liver (foie gras elaboration), and fat for cosmetic use (1,3,4).

In conclusion, some hematic and urinary physiological values from *R. catesbeiana*, were similar to those reported on other frogs (PCV, Na, K, Ca, Mg), and toads (total protein, albumin, alpha-1, alpha-2 and beta globulins, uric acid, triglycerides, glucose, P, Fe). In spite of the close phylogenic relationship between amphibians and birds, some parameters were quite different (RBC, hemoglobin, MCV, lymphocytes, creatinine, glucose, ALP, urinary density and sediment). Several frog blood values were similar to those found in human beings (ALT, GGT, bleeding and coagulation time), and both domestic monogastric (neutrophils, lymphocytes, LDL-C, Cl, LDH) and polygastric mammals (fibrinogen, AST).

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