FACENA, Vol. 19, pp. 57-66, 2003

REFERENCE INTERVAL AND PHYSIOLOGICAL VARIATIONS OF PLASMA ELECTROLYTES IN *RANA CATESBEIANA* (SHAW, 1802, AMPHIBIA: RANIDAE)

Norma B. COPPO [1]; José A. COPPO[2] y Santiago A. FIORANELLI[3]

RESUMEN: Intervalo de referencia y variaciones fisiológicas de electrolitos plasmáticos en *Rana catesbeiana* (Shaw, 1802, Anfibia: Ranidae).
Con el propósito de obtener valores normales para el ionograma plasmático e indagar modificaciones fisiológicas atribuibles al sexo, edad, peso, clima y sistemas de crianza y alimentación, se estudiaron 302 ejemplares sanos de *Rana catesbeiana*, de ambos sexos y edades de 9 a 21 meses, criados en el nordeste argentino. Se obtuvieron intervalos de confianza para sodio (116-121 meq/l), potasio (3,42-3,81 meq/l), cloro (100-116 meq/l), calcio (7,98-8,61 mg/dl), fósforo inorgánico (8,31-9,36 mg/dl), hierro (105-178 ug/dl) y magnesio (2,26-2,55 mg/dl). No se verificaron variaciones fisiológicas debidas al sexo, pero los avances de edad y peso correlacionaron con aumentos de potasio y disminuciones de calcio y fósforo inorgánico ($p < 0,05$). Los valores de magnesio, potasio, fósforo inorgánico y calcio fueron más bajos en invierno que en el resto de las temporadas del año. La técnica de manejo repercutió significativamente en el ionograma, cuyos valores fueron en general altos cuando el piso de los tanques poseía menor superficie cubierta por agua y *vice versa*. Los electrolitos plasmáticos fueron bajos en ranas mantenidas con vísceras, intermedios en la alimentación a base de pellets balanceados más larvas o lombrices, y altos en la alimentación "natural" en laguna. Se destaca la utilidad del ionograma para evaluar los estados nutricional y sanitario, proponiéndose su aplicación como instrumento idóneo para optimizar la producción de carne de rana.

ABSTRACT: To obtain plasma ionogram normal values, as well as sex, age, weight, climate, and breeding and feeding systems physiological variations, 302 samples of healthy *Rana catesbeiana* specimens from argentine northeastern (9-21 months old, 50-350 g liveweight, 50% each sex), were analyzed. Confidence intervals for sodium (116-121 meq/l), potasssium (3.42-3.81 meq/l), chloride (100-116 meq/l), calcium (7.98-8.61 mg/dl), inorganic phosphorous (8.31-9.36 mg/dl), iron (105-178 ug/dl) and magnesium (2.26-2.55 mg/dl), were obtained. Physiological variations due to sex were not verified. Weight and age advances correlated to potassium increase, and calcium and inorganic phosphorous decrease ($p < 0.05$). Magnesium, potassium, inorganic phosphorous and calcium values, were lower in winter than in remaining year seasons. Ionograma was significantly influenced by handling technique; in general, values were high when tanks floor had smaller surface covered by water, and *vice versa*. Plasma electrolytes were low in frogs maintained on viscera, intermediate when they fed on balanced pellets plus larvas or worms, and high when they fed "naturally" in a lagoon. Utility of ionogram to evaluate nutritional and sanitary states is

emphasized, and its application as useful instrument to optimize the frog production is recommended.

Key words: *Rana catesbeiana*, plasma electrolytes, normal values, physiological variations.

Palabras claves: *Rana catesbeian*, electrolitos plasmáticos, valores normales, variaciones fisiológicas.

---

(1) Profesor Adjunto, (2) Profesor Titular y (3) Becario Secretaría General de Ciencia y Técnica (SGCYT-UNNE). Cátedra de Fisiología General, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones (UNAM).
Azara 1552 (3300) Posadas, Misiones, Argentina. E-mail: jcoppo@vet.unne.edu.ar.

INTRODUCTION

Rana catesbeiana is autochtonous from North America and it is characterized by a great size, reaching the maturity to 12 months, with around 300 g liveweight (Longo, 1985; Lima and Agostinho, 1992). Their meat is eatable and it reaches high price because it contains scarce fat and it is almost exempt from cholesterol (Pavan, 1996). Nowadays, more than 200 bullfrog hatcheries exist in Argentina; habitually they are fed on similar balanced pellets to those elaborated for fish, because its true nutritional requirements are still unknown (Carnevia, 1995).

Amphibians are ectothermal animals and their metabolic rate, as well their growth rhythm, are regulated according to environmental temperature (Eckert, 1992; Wright *et al*., 1999). When temperature decreases to less than 10-15°C, frogs stop to feed and they become in lethargic life, using periovaric fatty bodies as energy source (Lima and Agostinho, 1992).

In frogs, electrolytes and water enter to organism through the skin and digestive tract, being eliminated by skin, urine, and feces; amphibians´ skin would be able to check the osmolarity of the surrounding liquid (Wilson, 1989). Fresh water frogs are hyperosmotic in their environment, reason why they tend to incorporate water by the skin and to decrease their corporal saline concentration (Hill, 1980; Goldstein, 1982). In these animals, high internal osmolarity (210-290 mOsm/L) and low external osmolarity (50 mOsm/L), would tend to provoke overhydration (entry of water by osmotic gradient) and loss of electrolytes (diffusion by concentration gradient). Homeostasis is achieved when the animal emits

abundant hypotonic urine and increases electrolytes tubular resorption and salt cutaneous absorption (Eckert, 1992).

Diverse illnesses can alter electrolytic homeostasis when perturbing the feedback of internal environment regulatory hormones (aldosterone, parathormone, calcitonin, vasopressin, thyroid, natriuretic), causing metabolic disturbances (Coles, 1989; Kaneko, 1989; Pesce and Kaplan, 1990; Uchiyama *et al*., 1998; Coppo, 2001). Body calcium accumulation decreases by calcitonin action and it increases by $1,25(OH)_2D_3$ action; parathormone effects would be scarce in *R.catesbeiana* (Baldwin and Bentley, 1980).

Calcium excess during metamorphosis early phases, would accelerate phenomenons like tail regression, intestine shortening, anterior legs growth, and epidermis keratinization, suggesting a probable thyroid activation (Menon *et al*., 2000). Mineral nutritional deficiencies are frequent in frog hatcheries, especially calcium lack, which provoke osseous malformations (Lima and Agostinho, 1992).

Classic texts of animal physiology have a lot of data about birds and mammals, but they have few references about frog internal environment. Such comparison would be important because amphibians would be phylogenetically more related with the birds than the mammals (Goldstein, 1982). Scarce data exist about frog plasma electrolytes physiological variations. Such parameters would be useful to evaluate health state in captive *R.catesbeiana*, which can suffer malnutrition, mineral lacks, hypo-hypervitaminosis, intestinal malabsorption, parasitosis, infections, endocrinopa-thies, intoxications, renal failures, skin and osseous disturbances, dehydration, acid-base imbalances, anemia, and stress (Fraser, 1986; Lima and Agostinho, 1992; Hecnar, 1995; Goldberg *et al*., 1998). All these illnesses can alter plasma electrolyte concentration, and they can impact negatively in frog commercial production. Determination of plasma electrolytes can assume diagnosis utility, whenever they have appropriate comparative reference intervals (Coppo, 2001).

The objective of this study was to obtain plasma electrolyte regional reference values from *R.catesbeiana*, as well as to verify eventual physiological variations due to sex, age, weight, climate (year season) and breeding and feeding systems.

MATERIALS AND METHODS

Experimental subjects, feeding and handling

Along two years of studies, a total of 302 healthy *R.catesbeiana* specimens were used. Two hundred seventy of them were maintained on intensive systems, in 3 different hatcheries from argentine northeastern. Samples from 90 frogs were taken in each breeding place, forming 12 groups of 7-8 animals every one (9 to 21 months old), 50% each sex, and 50 to 350 g liveweight. Thirty six percent of samples were taken in winter, and 64% in the remaining year seasons. Heating system during winter season was not employed in none of the hatcheries; all of them supplied food in a rate from 3-5% Iiveweight/day.

At the breeding place located in Oberá (Misiones), the water came from natural slopes and it occupied 25% of tanks surface; the food (diet 1) was commercial pellets balanced for fish (45% of protein), and it was supplied "dry" (scattered on the floor), sporadically accompanied by worms. Water from hatchery of Paso de la Patria (Corrientes) was underground, and it was extracted from the second layer by means of a perforation; it covered 50% of tanks floor, and frogs fed balanced pellets floating in the water (38% of protein), occasionally supplemented by flies larvas (diet 5). The hatchery of Jardín América (Misiones) also had emergent water, which occupied 90% of the tanks and it was used to give floating food. During the first year, batrachians ate (diet 4) a mixture of equal parts of bovine viscera (milled lung, 16% of proteins) and balanced pellets (45% of proteins), and during the second year such viscera were administered as unique food (diet 3).

The 32 remaining animals came from the hatchery mentioned in first term, but in this case the handling system was extensive (semicaptivity), because frogs were reared in a lagoon, where they selected exclusively "natural" foods (diet 2). They were mature animals (16-20 months old, both sexes), and samples were taken in winter and in the rest of year seasons.

Taking of samples

Frogs were transported to laboratory in thermal boxes, which contained a NaCl 0.6% isotonic solution cooled with

ice (2-3°C); this procedure produces desensitization and lethargy, facilitating the animal manipulations (Lima and Agostinho, 1992). Liveweight was obtained in an electronic balance Scientech-SL, with accuracy of 0.01 g. Samples were taken in morning hours (7-8 AM), after a 24 h fasting period. Blood was obtained by intracardiac puncture, carried out with syringe and needle. Sample was a venose and arterial blood mixture, because frogs, as anatomical characteristic, possess an unique ventricle (Goldstein, 1982). Blood was centrifuged to separate the clot and to obtain serum, before 2 h post-extraction.

Laboratory procedures

Plasma electrolytes were measured in a Labora Mannheim 4010 UV-visible spectrophotometer, using Wiener and Randox reagents, by means of habitual methods of laboratory (Pesce and Kaplan, 1990): chloride (mercuric tiocianate, 450 nm), calcium (cresolphtaleincomplexone, 570 nm), inorganic phosphorous (phosphomolyb-date, 620 nm), magnesium (calmagite, 520 nm) and iron (PBTS, 560 nm). Sodium and potassium were evaluated using Biopur reagents, in a Metrolab 305-D flame photo-meter.

Experimental design and statistical analysis

A completely randomized design was used. Independent variables were age, sex, weight, year season (climate), and feeding and handling system (according to origin). Dependent variables (quantitative continuous) were electrolyte values. The normality of the distribution 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 estimated by confidence intervals (CI±95%). After verifying variance homogeneity (Bartlett test), the analysis of the variance (ANOVA) was calculated by one way lineal model. Following the ANOVA ($p < 0.05$), the significance of differences was estimated by the Tukey test. Correlation coefficients were obtained by the Pearson procedure. All the calculations were made using the software *Statistix* Version 1996. For all inferences a 5% significance was specified, below which the equality null hypothesis was rejected.

RESULTS AND DISCUSSION

Descriptive statistics obtained for all studied amphibians are detailed in Table 1. Values approximately normal distributed (WS), allowed parametric statistics use. Confidence intervals were adjusted around arithmetic means, but individual ranges were wide.

Table 1: Electrolyte values obtained in studied total population (n = 302)

Parameter	$\bar{x}$ ± SD	WS	CI±95%	Range
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

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

Scarce regulation mechanisms and higher tolerance to hemodilution and hemoconcentration, would cause blood values great oscillation in frogs (Goldstein, 1982). This fact could explain the wide extent of ranges obtained in this trial. After food ingestion, changes in amphibian plasma composition would be registered (Busk *et al.*, 2000). Another changes would occur as consequence of circadian rhythm, caused by cortisol fluctuations (Wright *et al.*, 1999). Both postprandial and circadian effects were excluded of present study design, due respectively to previous fast and to basal condition of samples, because blood extraction was carried out in uniform morning hours.

Sodium plasma concentration in studied frogs was similar to those reported on *R.catesbeiana* (108±5 meq/L, Cathers 1997) and other frogs of fresh water (109 meq/L: Wilson, 1989 and 92-125 meq/L: Eckert, 1992). Natremia would be lightly higher on toads (128-139 meq/L: Coppo, 2001), birds (131-157 meq/L: Coles, 1989 and 130-146 meq/L: Coppo, 2001) and domestic mammals (132-156 meq/L: Kaneko, 1989; 110-155 meq/L: Coles, 1989 and 132-146 meq/L: Coppo, 2001).

Potassium level would be 2.4-6.7 meq/L (Coles, 1989; Kaneko, 1989) and 3.3-5.1 meq/L (Coppo, 2001) on mammals; 2.5-4.5 meq/L (Coles, 1989) and 5.1-6.4 meq/L (Coppo, 2001) on birds; 3.7-6.2 meq/L on toads (Coppo, 2001), and 2.6 meq/L (Wilson, 1989), 3 meq/L (Eckert, 1992) and

2.7±0.71 meq/L (Cathers, 1997) on frogs. Kalemia reported on amphibians was approximately similar to that found in this trial on *R.catesbeiana*.

Chloride value in studied frogs was lightly higher than those reported on frogs (70-98 meq/L: Eckert, 1992 and 77±6 meq/L: Cathers, 1997), and toads (85-96 meq/L: Coppo, 2001). On the other hand, it was similar to the values published on mammals (94-123 meq/L: Kaneko, 1989; 88-118 meq/L: Coles, 1989 and 93-112 meq/L: Coppo, 2001).

Great differences between calcium concentration on studied frogs and plasma calcium levels reported on the same species (8.05±0.88 mg/dL: Cathers, 1997), other frogs (8.4 mg/dL: Wilson, 1989 and 9.2 mg/dL: Eckert, 1992), toads and fowls (7.8-9.6 mg/dL and 9.3-10 mg/dL respectively: Coppo, 2001), and mammals (8-12 mg/dL: Coles, 1989; 6.2-13.6 mg/dL: Kaneko, 1989, and 8.4-11.5 mg/dL: Coppo, 2001), were not registered.

The value of inorganic phosphorous obtained in the present study was higher than those reported by Cathers (1997) on bullfrog (3.3±0.7 mg/dL). On the other hand, it was alike to those obtained on toads and birds (6.3-8.2 mg/dL and 6.2-8.7 mg/dL respectively: Coppo, 2001). Amphibians´ phosphatemia would be higher to those published on mammals (3-6 mg/dL: Kolb, 1987; 3-8 mg/dL: Coles, 1989; 2.6-6.9 mg/dL: Kaneko, 1989 and 3-5.2 mg/dL: Coppo, 2001).

In these frogs, plasma magnesium value was similar to those found on *R.catesbeiana* (2.05±0.35 mg/dL: Cathers, 1997), other fresh water frogs (3.1 mg/dL: Wilson, 1989 and 3.9 mg/dL: Eckert, 1992), toads and birds (2.3-4.2 mg/dL and 2-3 mg/dL respectively: Coppo, 2001), as well as on domestic mammals (2.5-3 mg/dL: Kolb, 1987; 1.8-3.7 mg/dL: Kaneko, 1989; 1.8-4 mg/dL: Coles, 1989 and 1-3 mg/dL: Coppo, 2001).

The iron level on studied frogs inserted in the reference interval reported on toads (83-145 ug/dL: Coppo, 2001), and domestic mammals (100-180 ug/dL: Kolb, 1987; 57-222 ug/dL: Kaneko, 1989; 86-193 ug/dL: Jain, 1993 and 93-165 ug/dL: Coppo, 2001). Iron and chloride data were enough to obtain central tendency and dispersion measures, but they were scarce to carry out the rest of the statistical studies, reason why they are omitted in the analysis of the variance and correlations.

Table 2: Electrolyte physiological variation according to age of frogs

Parameter	Age (months)											
	9	10	11	12	13	14	15	16	18	19	20	21
Na (meq/l)	129^{a}	124^{a}	113^{a}	126^{a}	116^{a}	115^{a}	117^{a}	103^{a}	117^{a}	122^{a}	109^{a}	111^{a}
K (meq/l)	2.7^{a}	3.2^{ab}	3.1^{ab}	3.3^{ab}	3.5^{ab}	3.4^{ab}	3.9^{ab}	3.8^{ab}	3.9^{ab}	4.2^{b}	4.3^{b}	4.2^{b}
Ca (mg/dl)	9.7^{a}	9.0^{a}	9.1^{a}	8.2^{b}	8.5^{b}	8.4^{b}	8.2^{b}	8.1^{b}	8.2^{b}	8.0^{b}	8.1^{b}	8.0^{b}
P (mg/dl)	10.9^{a}	10.5^{a}	10.9^{a}	9.2^{ab}	10.3^{a}	8.8^{b}	9.5^{ab}	8.6^{b}	6.9^{bc}	7.2^{bc}	6.4^{bc}	5.8^{c}
Mg (mg/dl)	2.3^{a}	2.2^{a}	2.8^{a}	2.5^{a}	2.2^{a}	2.0^{a}	1.9^{a}	2.6^{a}	2.8^{a}	1.9^{a}	2.9^{a}	2.7^{a}

Values expressed in arithmetic means. In each column, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 2 shows the attributable variations to frogs growth. Means comparison were significant for potassium, calcium, and inorganic phosphorous ($p < 0.05$), but they were not significant for sodium nor magnesium. While potassium revealed an increase trend when growth advancing, calcium and inorganic phosphorous showed opposed tendency, significantly decreasing when age progress. From end to end, relationship Ca/P increased from 0.8 (9 months) to 1.3 (21 months). Decreases of calcium and inorganic phosphorous are common in growing mammals (Koscinczuk *et al.*, 1988; Coppo, 2001).

Table 3: Electrolyte physiological variations according to sex and liveweight

Parameter	Sex		Liveweight (g)					
	male	female	50-99	100-149	150-199	200-249	250-299	300-349
Na (meq/l)	117^{a}	120^{a}	121^{a}	114^{a}	115^{a}	119^{a}	113^{a}	125^{a}
K (meq/l)	3.63^{a}	3.71^{a}	2.98^{a}	3.41^{ab}	3.66^{ab}	3.30^{ab}	4.21^{b}	4.13^{b}
Ca (mg/dl)	8.42^{a}	8.27^{a}	10.2^{a}	8.30^{b}	9.11^{ab}	8.06^{b}	7.83^{c}	8.22^{b}
P (mg/dl)	8.69^{a}	8.94^{a}	9.93^{a}	9.57^{a}	9.89^{a}	8.92^{ab}	7.42^{b}	6.96^{b}
Mg (mg/dl)	2.54^{a}	2.29^{a}	2.22^{a}	2.51^{a}	2.43^{a}	1.91^{a}	3.06^{a}	2.12^{a}

Values expressed in arithmetic means. In each column, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 3 indicates that differences between males and females ionogram were not significant. On the other hand, adult human calcium plasma concentration would be higher in men than in women (Coppo, 2001). In senility, increases of potassium and decreases of sodium, iron, and calcium, especially in women, would be registered (Coppo y Coppo, 2000). When weight rose, significant potassium values increases, and calcium and inorganic phosphorous decreases, were registered. These changes should be attributed to ontogenic reasons, since the amphibians' weights significantly rose when age increased, with high correlation ($r = 0.82$, $p = 0.02$).

Other correlations are detailed in Table 4. High lineal association degree between age advance, kalemia increase, and calcemia and phosphatemia decrease, was registered. Significant correlations between weight increase, potassium raise, and inorganic phosphorous decline, were also verified; calcemia decrease revealed a lineal association degree nearby to statistically significant one.

Table 4: Correlations between electrolytes, age, and liveweight

Parameter	Age			Liveweight		
	r	p	tendency	r	p	tendency
Na (meq/l)	-0.55	0.11	irregular	0.24	0.64	irregular
K (meq/l)	0.96	0.0001	↑	0.86	0.02	↑
Ca (mg/dl)	-0.82	0.001	↓	-0.74	0.08	↓
P (mg/dl)	-0.97	0.0001	↓	-0.92	0.009	↓
Mg (mg/dl)	0.26	0.41	irregular	0.08	0.87	irregular

r: correlación (Pearson), p: significance.

Table 5 reveals that, excepting the sodium case, plasma ions were significantly lower in winter than in remaining year seasons. Such fact could be due to feeding suppression (winter lethargy), but also to effects of cold. Electrolyte regulatory hormones release could be altered by low environmental temperature (Wright *et al*., 1999). On amphibians, the cellular membranes polarization, which depends on the electrolytes concentration, would change due to cold (Dalo *et al*., 1995).

Decrease of sodium, potassium, calcium, chloride, sulfate and protein concentrations in amphibian plasma, would be produced by cold. This phenomenon is attributed to an overhydration state, verified by inulin space increase. When temperature decreases from 30 to 10°C, total plasma osmolarity would decrease 5% in *R. catesbeiana* and 14% in *Bufo marinus*. Such ionic changes would be an important mechanism of pH adjustment in the face of temperature changes (Stinner and Hartzler, 2000).

Table 5: Physiological variations according to climate

Parameter	Season	
	Winter	Rest of year
Na (meq/l)	117.3[a]	120.5[a]
K (meq/l)	3.21[a]	4.02[b]
Ca (mg/dl)	7.63[a]	9.22[b]
P (mg/dl)	7.83[a]	9.65[b]

Mg (mg/dl)	1.92^{a}	3.03^{b}

Values expressed in arithmetic means. In each column, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 6 shows that lowest electrolyte levels were registered in frogs fed on viscera (diet 3). Plasma mineral concentrations were also low in animals fed on diet 4 (viscera plus balanced pellets). Highest sodium, potassium, calcium and magnesium values were verified in frogs whose food was obtained "naturally" in the lagoon (diet 2) and highest inorganic phosphorous values were registered in animals fed on balanced pellets plus worms (diet 1). These results suggest that type of food would condition the level of plasma electrolytes.

Mature frog alimentary tract would be adapted to digest insects, annelids, crustaceans, mollusks, and small vertebrates (Hill, 1980; Eckert, 1992). In argentine northeastern, natural diet of terrestrial anurous like *Bufo* sp. would be mainly compounded by coleopterons and hymenopterans (Duré and Kehr, 1999). In the present study, necropsies made on frogs fed naturally in the lagoon allowed to verify that alimentary tract of frogs contained small fish, other frogs and tadpoles, crabs, and aquatic myriapods, coleopterons and hemipterans, as well as abundant grass.

Table 6: Physiological variations according to food type (n=302) and hatchery handling (n=270)

Parameter	Type of food					Type of handling		
	1	2	3	4	5	Oberá	J.América	P. Patria
Na (meq/l)	128^{a}	130^{a}	108^{b}	112^{b}	120^{ab}	129^{a}	109^{b}	120^{a}
K (meq/l)	3.83^{a}	3.92^{a}	3.03^{b}	3.19^{b}	3.85^{a}	3.80^{a}	3.46^{a}	3.59^{a}
Ca (mg/dl)	8.73^{a}	9.25^{a}	7.24^{c}	8.16^{b}	8.50^{ab}	8.73^{a}	7.74^{b}	8.55^{ab}
P (mg/dl)	10.4^{a}	9.75^{a}	6.82^{b}	7.14^{b}	9.73^{a}	10.1^{a}	8.13^{b}	8.51^{ab}
Mg (mg/dl)	2.51^{a}	2.68^{a}	2.02^{b}	2.39^{a}	2.44^{a}	2.78^{a}	2.02^{b}	2.41^{c}

Values expressed in arithmetic means. Diet 1: balanced (protein 45%) plus worms, 2: natural (lagoon), 3: viscera (protein 16%), 4: balanced (protein 45%) plus viscera, 5: balanced (protein 38%) plus larvas. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

Electrolyte values found in this trial would suggest that frogs reared in semi-captivity way (lagoon), would obtain higher mineral quantities than those fed artificially. Unfortunately, when *R.catesbeiana* settles in

some lagoon, it would be considered ecologically as "undesirable guest", because due to its voracious appetite, original aquatic fauna could be rapidly extinguished (Longo, 1985; Lima and Agostinho, 1992). Necropsies allowed to confirm that cannibalism would not be unusual in this species (Longo, 1985).

Attributable significant differences to conditions of hatcheries handling, were also registered (Table 6). Mineral rates were higher in frogs coming from Oberá and Paso de la Patria (bigger dry floor surface), and they were lower in the samples coming from Jardín América (bigger floor surface covered by water).

Proportion of tanks floor covered by water would be an important handling factor in frogs hatcheries, because electrolytes and gases exchange is carried out through the skin. At high temperatures, O_2 intake by skin would be higher than O_2 intake made by lungs, and *vice versa* (Goldstein, 1982). Some frogs would hibernate submerged, exchanging O_2 and CO_2 exclusively through the skin; *Frog esculenta* would be able to survive submerged during 2-3 weeks. On the other hand, *R.catesbeiana* would have scantly developed this mechanism and it would not survive much time, maybe due to its great size (Hill, 1980).

Samples of frogs with presumptive health state deterioration were taken in several occasions, although such values were excluded of the statistics. Symptoms as adynamia, weakness, anorexy, dehydration, diminished weight and skin abnormal coloration were related to low concentration of calcium (5.7 mg/dl), as well as to high concentration of sodium (185 meq/l), potassium (7 meq/l), chloride (151 meq/l), calcium (12.8 mg/dl), inorganic phosphorous (23 mg/dl), iron (240 ug/dl) and magnesium (6.08 mg/dl).

One of the current priorities in frogs production is to evaluate scientifically the food type which will be provided because, until the moment, formulations conceived for carnivorous fishes as trouts, are being used empirically by producers. Studies which relate food type with internal environment metabolic and nutritional indicators, should be carried out (Carnevia, 1995). Investigations to establish the real nutritional requirements of this frog, are necessary to optimize the cost/benefit relationship (Longo, 1985; Lima and Agostinho, 1992). Verified alterations in sick animals suggest that ionogram changes could also be effective indicators of sanitary and metabolic dysfunctions on

amphibians, just as it occurs in other species (Coles, 1989; Kaneko, 1989; Pesce and Kaplan, 1990).

In conclusion, a plasma electrolytes reference interval, characterized by wide ranges, was obtained on bullfrog reared in northeastern argentine. Although there were not significant differences between sexes, physiological variations were verified according to age, weight, climate, and handling and feeding systems.

ACKNOWLEDGEMENTS

To the producers from Oberá, Jardín América and Paso de la Patria hatcheries, for their valuable collaboration. To Wiener Lab for supply the reagents used in the trial. To Lic. Noelia Barboza for the English revision.

BIBLIOGRAPHY

BALDWIN, G.F. and P.J. BENTLEY, 1980. Calcium metabolism in bullfrog tadpoles (*Rana catesbeiana*). *J. Exp. Biol.*, 88: 357-365.

BUSK, M.; F.B. JENSEN and T. WANG, 2000. Effects of feeding on metabolism, gas transport, and acid-base balance in the bullfrog, *Rana catesbeiana*. *Am. J. Physiol.*, 278: 185-195.

CARNEVIA, D., 1995. Ranicultura, estado actual de la explotación y comercialización de ranas para consumo. *Anales de las VIII Jornadas Veterinarias de Corrientes*, Argentina: 81.

CATHERS, T., 1997. Serum chemistry and hematology for anesthetized american bullfrogs. *J. Zoo & Wildlife Med.*, 28: 171-174.

COLES, E.H., 1989. *Veterinary Clinical Pathology*. 4th ed., Saunders, Philadelphia. 486 p.

COPPO, J.A. y N.B. COPPO, 2000. Evolución de valores de la bioquímica clínica conforme avanza el envejecimiento. *Prensa Méd. Arg.*, 87: 717-727.

COPPO, J.A., 2001. *Fisiología Comparada del Medio Interno*. Dunken, Buenos Aires. 297 p.

DALO, N.L.; J.C. HACKMAN and R.A. DAVIDOFF, 1995. Motoneuron depolarizations, paroxysmal activity, and reflex changes induced by rapid cooling of the toad spinal cord. *Comp. Biochem. & Physiol.*, 112: 517-525.

DURÉ, M.I. y A.I. KEHR, 1999. Explotación diferencial de los recursos tróficos en cuatro especies de bufonidos del nordeste argentino. *Actas Cienc. & Técn. UNNE*, 6: 17-20.

Eckert, R., 1992. *Animal Physiology*. 3rd ed., Freeman, New York. 683 p.

Fraser, C.M., 1986. *The Merck Veterinary Manual*. Merck Inc., Rahway. 2092 p.

Goldberg, S.R.; C.R. Bursey and H. Cheam, 1998. Helminths of two native frog species (*Rana chiricahuensis*, *Rana yavapaiensis*) and one introduced frog species (*Rana catesbeiana*) from Arizona. *J. Parasitol.*, 84: 175-177.

Goldstein, L., 1982. *Comparative Physiology*. Saunders, Philadelphia. 454 p.

Hecnar, S.J., 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario. *Environ. Toxicol. & Chem.*, 14: 2131-2137.

Hill, R.W., 1980. *Fisiología Animal Comparada*. Reverté, Barcelona. 610 p.

Jain, N.C., 1993. *Essentials of Veterinary Hematology*. Lea & Febiger, Philadelphia. 417 p.

Kaneko, J.J., 1989. *Clinical Biochemistry of Domestic Animals*. 4th ed., Academic Press, San Diego.832 p.

Koscinczuk, P.; A. Beneventano and J.A. Coppo, 1989. Radiological and biochemical evaluation of bone growth in young dogs. *Acta Physiol. Pharm.*, 39: 289-297.

Lima, S.L. y C.A. Agostinho, 1992. *A Tecnología de Criaçao de Rás*. Impr. Universitaria, Vicosa. 288 p.

Longo, A.D., 1985. *Manual de Ranicultura*. Icone, São Paulo. 317 p.

Menon, J.; E. Gardner and S. Vail, 2000. Developmental implications of differential effects of calcium in tail and body skin of anuran tadpoles. *J. Morphol.*, 244: 31-43.

Pavan, M., 1996. Carne de rana toro. El batracio versus la merluza. *Vet. Arg.*, 13: 741-742.

Pesce, A.J. and L.A. Kaplan, 1990. *Methods in Clinical Chemistry*. Mosby, Saint Louis. 1380 p.

Stinner, J.N. and L.K. Hartzler, 2000. Effect of temperature on pH and electrolyte concentration in air-breathing ectotherms. *J. Exp. Biol.*, 203: 2065-2074.

Uchiyama, M.; T. Takeuchi and K. Matsuda, 1998. Effects of homologous natriuretic peptides in isolated skin of the bullfrog, *Rana catesbeiana*. *Comp. Biochem. Physiol.*, 120: 37-42.

Wilson, J.A., 1989. *Principles of Animal Physiology*. McMillan, New York. 984 p.

Wright, M.L.; B.A. Rzasa; R.J. Weir and A.M. Babski, 1999. Influence of cortisol on the larval bullfrog thyroid axis *in vitro* and *in vivo* and on plasma and ocular melatonin. *Gen. Comp. Endocrinol.*, 116: 249-260.

Recibido/Received/: 10-Jun-03
Aceptado/Accepted/: 23-Oct-03