Physiological variation of enzymatic activities in blood of bullfrog, *Rana catesbeiana* (Shaw, 1802)*

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Abstract

Coppo, J.A.; Mussart, N.B.; Fioranelli, S.A.: ***Physiological variations of enzymatic activities in blood of bullfrog, Rana catesbeiana (Shaw, 1802).*** The aim of this study was to determine plasma enzymogram reference values in bullfrog, *Rana catesbeiana*, to correlate them with physiological modifications attributable to sex, age, weight, climate, and breeding and feeding systems. For this purpose, three hundred and two healthy animals (from 9 to 21 months–old, both sexes) were used. Reference intervals for alkaline phosphatase, ALP (157±32 IU/L), alanine aminotransferase, ALT (12.4±2.6 IU/L), aspartate aminotransferase, AST (48.1±9.3 IU/L), gammaglutamyl transferase, GGT (9.2±1.6 IU/L), lactate dehydrogenase, LDH (117±22 IU/L), butyryl cholinesterase, CHE (168±32 IU/L) and creatine phosphokinase, CPK (432±85 IU/L), were obtained. Growth showed a correlation to weight increase ($r = 0.82$, $p = 0.02$). Significant lineal association between the age increment and ALP decrease (9 months = 196 versus 21 months = 102 IU/L), as well as the increases of CHE (126 versus 226 IU/L) and CPK (280 versus 572 IU/L), were registered. ALP and CPK were higher in males than females ($p<0.05$). Except GGT, enzymatic activities were higher in winter than on the other seasons. ALP, AST, GGT and LDH values were significantly higher when water covered 90% of the tank floor and food was administered as floating pellets. On the contrary, values were low when water covered only 25% of the tank and pellets were given on the floor. The highest values on hepatic strain marker enzymes (ALP, ALT, AST, GGT and LDH) were registered in frogs which have eaten bovine lung, with or without balanced pellets, and the lowest ones were verified on animals fed naturally in a lagoon. The usefulness of enzymogram to evaluate sanitary, metabolic, and nutritional state is proposed, while its application as suitable tool to improve frog meat production is emphasized.

Key words: *Rana catesbeiana*, plasma enzymes, physiological variations.

INTRODUCTION

Cells contain enzymes that are necessary to their function. When the integrity of a cell is disrupted, enzymes escape into plasma, where their activity can be measured as an useful index of cell integrity. Demonstration that specific plasma enzyme activity increases or decreases with disease, encourage researchers to evaluate a variety of enzyme systems looking for those organ or tissue specific. Nowadays, enzymogram plays an important role in diagnosis and prognosis of animal disease [5].

Modifications in enzyme activity occur by cell death, increase or decrease enzyme production, obstruction of normal excretory route, increase cell membrane permeability, or impair circulation [15].

Chronic hepatic disorders and excessive steroids result in increase plasma alkaline phosphatase (ALP) in most animals. During normal bone growth in young animals, a large amount of ALP is in plasma; osteopathies also result in increase plasma ALP. The liver specificity of gammaglutamyl transferase (GGT) has been recently found and, for this reason, is usual as an hepatobiliary disease marker. Increase plasma aspartate aminotransferase (AST) is associated with cell necrosis of liver and skeletal or cardiac muscle, starvation, and lacking vitamin E. Plasma alanine aminotransferase (ALT) is an acute hepatic damage good marker. Skeletal and cardiac muscle damage results in great increase of plasma creatine phosphokinase (CPK). Brain also contains large amounts of the last one. Males have about 50% more CPK activiy than females. Lactate dehydrogenase (LDH) is released to liver, lung, muscle, heart and kidney tissue after cellular damage. Plasma cholinesterase is a pseudocholinesterase (butyryl cholinesterase, CHE), which is made in liver, pancreas, intestinal mucosa and brain; decreases in CHE have been reported in liver

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failure, muscular dystrophy, chronic renal disease and organophosphate insecticide intoxication [5, 6, 15].

Many enzymes register important physiological variations due to age (growth, aging), sex, genital stage (gestation, nursing), diet, physical exercise and other variables [6]. Reports about normal values and physiological variations from *Rana catesbeiana* enzymogram are scarce. Bullfrog is originate from North America and is characterized by a great size, reaching the maturity in 12 months, with approximately of 300 g liveweight [16, 17]. Its meat is good taste and reaches high price because its scarce fat content and almost cholesterol free [18].

Amphibians are ectothermal animals and their metabolic rate, as well as their growth rhythm, are regulated to environmental temperature [21, 25]. When temperature decreases less than 10–15ºC, frogs stop feeding and they become in lethargic state, using periovaric fatty bodies as energy source [16].

Amphibian enzymes should be studied in different physiological conditions, because in ectothermal animals the cold adaptation is carried out by means of enzymatic activity increase or substrate affinity decrease [23]. Main enzymes that increase by low temperatures correspond to glycolytic pathway, tricarboxylic acid cycle, and cytochrome system; on the other hand, acetylcholinesterase modifies its substrate affinity [12]. Arginase activity in hepatic and renal tissues varies according to temperature and hydration degree, because in dehydration it is necessary to excrete urea, and when humitidy is abundant it is convenient to eliminate ammonia [7].

Underfeeding, inadequate handling and transmissible diseases, are the main health problems in captivity reared bullfrog, which also can suffers varied intoxications [16, 17]. Bullfrog enzymogram values reference interval, as well as their physiological variations, would be useful to elucidate metabolic, nutritional and sanitary dysfunctions, which can affect frog meat production.

The objective of this study was to obtain reference values from *Rana catesbeiana* plasma enzymatic activities, as well as to verify attributable physiological variations to sex, age, weight, climate (year season), and rearing and feeding systems.

MATERIAL AND METHODS

Experimental subjects, feeding and handling. Following two years of studies, a total of 302 healthy *Rana catesbeiana* specimens were used. Two hundred seventy of them were bred on intensive systems, in 3 different hatcheries from argentine northeastern. Samples from 90 frogs were taken in each breeding place, arranged in 12 groups of 7–8 animals each one, between 9 to 21 months, approximately 50% males and 50% females, from 50 to 350 g of liveweight. Thirty six percent of samples were taken in winter, and 64% in the rest of the year. Heating system during winter season was not employed in any hatchery, and all of them supplied food in a rate from 3–5% liveweight/day.

At the breeding place of Oberá (Misiones), the water came from natural slopes and occupied 25% of tanks surface; the food (diet 1) was balanced commercial pellets for fish, with 45% of protein content, 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 on water, with 38% of protein content, occasionally supplemented by flies larvae (diet 5). The hatchery of Jardín América (Misiones) also had emergent water, which occupied 90% of the tanks and was used to give floating food. During the first year, batrachians ate a mixture of equal parts of bovine milled lung, with 16% of protein content, and balanced pellets with 45% of protein content (diet 4). During the second year such viscera were given as unique food (diet 3).

The 32 remaining animals came from the Oberá hatchery, but in this case the handling system was extensive (semicaptivity), because frogs were reared in a lagoon, and 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 the year.

Sampling. Frogs were carried to laboratory in thermal boxes with 0.6% NaCl isotonic solution ice cooled (2–3ºC); this procedure produces desensitization and lethargy, allowing animal handling [16]. Liveweight was obtained in an electronic balance Scientech–SL, with accuracy of 0.01 g. Samples were taken early in the morning (7–8 AM), after a 24 h fasting period. Blood was obtained by intracardiac puncture, carried out with syringe and needle. Frogs possess and unique ventricle [11], so sample was venose and arterial blood mixture. Blood was centrifuged to separate serum, no more than 2 h after extraction.

Laboratory procedures. By a Labora Mannheim 4010 UV–visible spectrophotometer, using disposable semi–micro cuvettes of 10 mm light path and Wiener Lab reagents, activities of alkaline phosphatase (ALP, phenylphosphate technique, measured at 520 nm), alanine aminotransferase (ALT, oxoglutarate–NADH, 340 nm), aspartate aminotransferase (AST, aspartate–ketoglutarate, 505 nm), gammaglutamyl transferase (GGT, p–nitroanilide kinetic method, 405 nm), lactate dehydrogenase (LDH, dinitrophenylhydrazine, 505 nm), butyryl cholinesterase (CHE, kinetic with butyrilthiocholine, 405 nm) and creatine phosphokinase (CPK, creatine–ATP, 620 nm), were measured [14, 19].

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 enzymogram values, expressed in International Units

by liter (IU/L). The normality of the distribution was assessed using Wilk–Shapiro test (WS). Parametric descriptive statistics included measures of central tendency (arithmetic mean, $\bar{x}$), dispersion (standard deviation, SD) and ranks. Fiduciary probability was estimated by confidence intervals (CI±95%). After verifying variance homogeneity (Bartlett test), the analysis of variance (ANOVA) was calculated by one way lineal model. Following ANOVA ($p < 0.05$), the significance of differences was estimated by Tukey test. Correlation coefficients were obtained by Pearson procedure. All calculations were made using *Statistix* software Version 1996. Equality null hypothesis was rejected under 5% of specified significance for all inferences.

Table 1. *Enzymatic activities obtained in studied total population (n = 302).*

enzyme	$\bar{x}$ ± SD	WS	CI±95%	rank
ALP	157 ± 32	0.959	144 – 170	73 – 248
ALT	12.4 ± 2.6	0.941	10.0 – 14.8	7 – 20
AST	48.1 ± 9.3	0.947	42.8 – 53.4	23 – 80
GGT	9.2 ± 1.6	0.932	7.8 – 10.6	5 – 20
LDH	117 ± 22	0.940	99 – 135	50 – 260
CHE	168 ± 32	0.975	151 – 185	45 – 274
CPK	432 ± 85	0.937	365 – 500	156 – 919

Values expressed in International Units by liter (IU/L). $\bar{x}$: arithmetic mean, SD: standard deviation, WS: Wilk–Shapiro distributive normality test (chart coefficient: 0.947, $\alpha = 0.05$), CI±95%: 95% confidence interval.

Table 2. *Enzymatic activities physiological variation according to age of frogs (n = 302).*

age (months)	ALP	ALT	AST	GGT	LDH	CHE	CPK
9	196^{a}	12^{a}	59^{a}	7^{a}	138^{a}	126^{a}	280^{a}
10	191^{a}	17^{a}	65^{a}	13^{a}	120^{a}	134^{a}	318^{a}
11	175^{ab}	16^{a}	53^{a}	9^{a}	160^{a}	134^{a}	302^{a}
12	180^{a}	12^{a}	75^{a}	10^{a}	125^{a}	136^{a}	346^{a}
13	178^{ab}	13^{a}	51^{a}	7^{a}	150^{a}	132^{a}	335^{a}
14	183^{a}	12^{a}	59^{a}	5^{a}	126^{a}	181^{b}	419^{ab}
15	159^{b}	14^{a}	45^{a}	11^{a}	129^{a}	168^{b}	422^{ab}
16	134^{bc}	8^{a}	52^{a}	7^{a}	87^{a}	170^{b}	458^{b}
18	141^{bc}	9^{a}	41^{a}	9^{a}	107^{a}	182^{b}	580^{bc}
19	123^{c}	8^{a}	27^{a}	9^{a}	72^{a}	204^{c}	557^{bc}
20	99^{d}	8^{a}	30^{a}	12^{a}	70^{a}	217^{c}	618^{c}
21	102^{d}	9^{a}	39^{a}	8^{a}	93^{a}	226^{c}	572^{bc}

Arithmetic means expressed in International Units by liter (IU/L). In each column, different letters indicate significant differences (Tukey test, $p < 0.05$).

RESULTS

Descriptive statistics obtained from all studied amphibians are detailed in Table 1. Approximately normal distributed values (WS), allowed parametric statistics use. Confidence intervals were adjusted around arithmetic means, but individual ranks were wide.

Age attributable variations are shown in Table 2. ALP, CHE and CPK significant modifications were registered with growth. CHE and CPK revealed a clear increase trend, meanwhile ALP evolution was decreasing. ALT, AST and LDH differences were not significant, nevertheless they revealed a declining tendency, which were inversely proportional to age increase. Irregular changes with scarce oscillations, without defined tendency, were registered for GGT.

Significant differences between sex were registered only for ALP and CPK activities, which were higher in males than in females (Table 3). Variations due to frogs liveweight were significant for ALP activity, which decreased when the weight increased, and CPK, which augmented with the weight. Correlation between age and weight was significant ($r = 0.82$, $p = 0.02$).

Other lineal associations verified between enzymatic activities, age and liveweight, are shown in Table 4. Age increase correlated negatively with ALP and LDH (descendent) and positively with CHE and CPK (ascendent), significantly in both cases. Lineal association between weight increase and CPK rise was also significant. Correlation between ALP decrease and weight increase assumed a probability very near to the significant one ($p = 0.06$).

Table 5 reveals that, except GGT, enzymatic concentrations were higher in winter than in the remaining year seasons, significantly for ALP, AST, LDH, CHE and CPK.

Table 3. *Enzymatic activities physiological variations according to sex and weight (n = 302).*

enzyme	sex		liveweight (g)					
	males	females	50–99	100–149	150–199	200–249	250–299	300–349
ALP	168^{a}	146^{b}	186^{a}	138^{b}	165^{c}	152^{bc}	156^{bc}	126^{d}
ALT	11.3^{a}	12.5^{a}	12.5^{a}	12.0^{a}	13.5^{a}	13.2^{a}	11.3^{a}	11.0^{a}
AST	46.7^{a}	48.9^{a}	38.8^{a}	50.5^{a}	51.1^{a}	39.2^{a}	54.3^{a}	43.8^{a}
GGT	8.8^{a}	10.1^{a}	8.3^{a}	10.1^{a}	10.4^{a}	7.2^{a}	9.7^{a}	8.5^{a}
LDH	132^{a}	104^{a}	144^{a}	128^{a}	98^{a}	92^{a}	116^{a}	107^{a}
CHE	173^{a}	165^{a}	147^{a}	160^{a}	181^{a}	192^{a}	135^{a}	183^{a}
CPK	498^{a}	405^{b}	375^{a}	409^{a}	440^{b}	379^{a}	483^{b}	511^{c}

Arithmetic means expressed in International Units by liter (IU/L). In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

Differences attributable to hatchery handling system and type of food consumed by frogs, are detailed in Table 6. No significant differences in CHE and CPK activities were registered. The handling system in which the water occupied 90% of the tanks floor and food was supplied floating in the water, registered significantly higher values of ALP, AST, GGT and LDH. Lowest values were registered in the hatchery in which the water covered only 25% and food was given on floor (dry).

ALP, ALT, AST, GGT and LDH values were significantly higher in frogs which consumed bovine viscera (as unique food or associated with balanced pellets). On the other hand, significantly lowest values were verified in frogs fed naturally in the lagoon. In this group, necropsies 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. Remaining diets produced intermediate plasma values.

Samples of frogs with presumptive health state deterioration were taken in several occasions, although such values were excluded for the statistics. Symptoms as adynamia, weakness, anorexy, dehydration, diminished weight and skin abnormal coloration were related with high enzymatic activities of ALP (till 568 IU/L), ALT (55 IU/L), AST (450 IU/L), GGT (36 IU/L), LDH (650 IU/L) and CPK (3300 IU/L), as well as with decreases of CHE (45 IU/L).

Table 4. *Correlations obtained between enzymatic activities, age, and liveweight.*

	age (months)			liveweight (g)		
	r	p	tendency	r	p	tendency
ALP	–0.95	0.0001	↓	–0.72	0.06	↓
ALT	–0.59	0.08	irregular	–0.52	0.28	irregular
AST	–0.54	0.12	irregular	0.19	0.70	irregular
GGT	0.33	0.26	irregular	–0.15	0.78	irregular
LDH	–0.80	0.001	↓	–0.62	0.18	irregular
CHE	0.95	0.0001	↑	0.27	0.59	irregular
CPK	0.97	0.0001	↑	0.80	0.05	↑

r: correlation coefficient (Pearson), p: significance ($p < 0.05$, $n = 302$).

Table 5. *Enzymatic activities physiological variations according to climate.*

	season	
	winter	rest of year
ALP	171[a]	144[b]
ALT	14.2[a]	11.4[a]
AST	58.6[a]	39.3[b]
GGT	8.4[a]	11.1[a]
LDH	138[a]	94[b]
CHE	177[a]	156[b]
CPK	447[a]	419[b]

Arithmetic means expressed in International Units by liter (IU/L). In each line, different letters indicate significant differences (Tukey test, $p < 0.05$, $n = 302$).

DISCUSSION

Scarce regulation mechanisms and high tolerance to hemodilution and hemoconcentration, would cause blood values variation in frogs [11]. This fact could explain the wide extent of ranks obtained in this trial. Changes in amphibian plasma composition would be registered after food ingestion [2]. Other changes would occur as consequence of circadian rhythm, caused by cortisol fluctuations [25]. Both postprandial and circadian effects were excluded from this study design, owing previous fast and basal condition of samples respectively, because blood extraction was carried out in uniform morning hours.

Bibliography about frog enzymatic values is very scarce. In 11 anesthetized *Rana catesbeiana* specimens, from 289 to 468 g liveweight, values of LDH (33±20 IU/L) were lower than those obtained in this study [4]; the anesthesia effect or the employment of another technique for enzymatic assay (not specified), could be the cause of such differences. Studied amphibians registered values of ALP, ALT, GGT and LDH, similar to those admitted in human being reference interval, as well as lower CHE activities, and higher AST and CPK concentrations [6, 14, 19].

Amphibians would be phylogenetically more related with birds than mammals [7, 11]; however, the frog ALP activity resulted lower than those habitually found in bird

Table 6. *Enzymatic activities physiological variations according to hatchery handling (n = 270) and food type (n = 302)*

enzyme	handling system			type of food				
	Oberá	J. América	P. Patria	1	2	3	4	5
ALP	143[a]	177[b]	148[a]	151[a]	135[b]	168[a]	177[a]	165[a]
ALT	12.1[a]	13.0[a]	12.9[a]	12.0[a]	10.4[a]	13.1[a]	15.1[a]	12.2[a]
AST	43.5[a]	55.2[b]	47.9[a]	47.5[a]	40.8[b]	57.2[c]	48.3[a]	47.4[a]
GGT	8.7[a]	11.3[b]	8.9[a]	9.1[a]	7.4[a]	11.7[b]	9.2[a]	7.5[a]
LDH	103[a]	141[b]	111[a]	104[a]	95[a]	139[b]	126[ab]	119[ab]
CHE	176[a]	160[a]	169[a]	166[a]	182[a]	201[a]	153[a]	148[a]
CPK	450[a]	413[a]	438[a]	474[a]	427[a]	438[a]	376[a]	454[a]

Arithmetic means expressed in International Units by liter (IU/L). Diet 1: balanced (proteins 45%) plus worms, 2: natural (lagoon), 3: viscera (proteins 16%), 4: balanced (proteins 45%) plus viscera, 5: balanced (proteins 38%) plus larvae. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

plasma [5, 6]. Amphibians of the present study showed approximately similar values of ALP, ALT, GGT and LDH, but higher values of CPK, than those reported for most of domestic mammals [15]. Frog AST values were similar to those published for ruminant, but they were higher than those found in canine, and lower than those reported for equine. CHE activity was lightly lower than those found in ruminant, and very much lower than those admitted for canine and equine [5, 6, 22].

Increase of age caused significant modifications in some enzymatic activities. Rise of CPK could be due to muscular mass increment produced by growth, just as it would occur in other species [6], because the source of this enzyme is mainly muscular [5, 15, 19]. In view of the abundance of ALP in amphibian bones [10], decreases of this enzyme could be attributed to osseous ALP isoenzyme declining, just as it happens in other species when osseous development advances [5, 6, 15]. During the child growth, ALP activity would be four times higher than in adults [14, 19].

Other enzymatic activities would be also affected by growth. Tadpoles (stages X to XVII) would be ammoniotelic and they would have scarce activities of carbamylphosphatesynthase, ornithinecarbamyltransferase, argininesynthase and arginase. In stages XX to XXIV (metamorphosis) they would become ureotelic and enzymatic activities of ornithine cycle would rise [12, 24].

In the same sense, ALP and CPK activities could have been higher in males than in females, due to higher osseous (ALP) and muscular (CPK) male development, just as it occurs in most vertebrates [6]. Decrease of ALP and increase of CPK provoked by weight rise should be attributed to age advance, in view of the significant lineal association registered between weight and age.

Increase of plasma enzymes registered in frogs during the winter, coincides with reports which assert that amphibians, as a cold adaptation mechanism, would be able to increase their enzymatic activities when environmental temperature decreases [23]. *Rana catesbeiana* metabolic rate would be regulated according to environmental temperature [9, 21], mainly by means of hormonal variations [25].

The proportion of water in tanks would be an important handling factor in frogs hatcheries, because gases and electrolytes exchange is carried out through the skin. Studies on *Bufo americanus* indicated that, at high temperatures, O_2 intake by skin would be higher than O_2 intake made by lungs, and *vice versa*; on the other hand, for CO_2 excretion, skin would be always more important, in any temperature [11]. Seasonal climatic changes would generate amphibian cardiorespiratory adaptation responses and they would markedly affect the O_2 consumption, as well as the concentration of some plasma components [1, 20]. In hypoxia, metabolism strays towards anaerobic way, and higher LDH availability is necessary for the interconversion from lactate to pyruvate [15]. 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, *Rana catesbeiana* would have scantly developed this mechanism and would not survive much time, probably to its big size [12].

In frogs fed with bovine viscera, high values of ALP, ALT, AST, GGT and LDH (preponderately hepatic enzymes) could indicate an hepatopancreas overload, perhaps because it would be an inappropriate diet for amphibians [5, 6, 22]. Supporting this hypothesis, the lowest plasma enzymatic activities were registered in animals which could select its natural food in a lagoon. Although tadpoles would behave as vegetarians [11, 24], and they would register certain coprophagy degree in hatcheries [13], the mature frog alimentary tract would be adapted to digest insects, annelids, crustaceans, mollusks, and small vertebrates [9, 12]. In northeastern Argentina, natural diet of terrestrial anurous like *Bufo sp* would be mainly compound by coleopterons and hymenopterans [8]. Unfortunately, when *Rana catesbeiana* settles in some lagoon, it would be considered ecologically an "undesirable guest", because original aquatic fauna would be rapidly extinguished due to its voracious appetite [16, 17]. Necropsies allowed confirm that cannibalism would not be unusual in this species [17].

One of the current priorities in frog production is to scientifically evaluate the type of food which will be provided because, to the present, formulations conceived for carnivorous fish as trouts, are being used empirically by producers. Studies which relate food type with internal environment metabolic and nutritional indicators, should be carried out [3]. Investigations to establish the real nutritional requirements of this frog, are necessary to optimize the cost/benefit relationship [16, 17]. Alterations verified in sick animals suggest that enzymogram changes could also be effective indicators of sanitary and metabolic amphibians dysfunctions, just as it occurs in other species [5, 14, 15, 22].

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Resumen

Coppo, J.A.; Mussart, N.B.; Fioranelli, S.A.: ***Variaciones fisiológicas de actividades enzimáticas en sangre de Rana catesbeiana (Shaw, 1802).*** Con el propósito de obtener valores normales para el enzimograma 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. Se obtuvieron intervalos de referencia para fosfatasa alcalina, ALP (157±32 UI/L), alanin aminotransferasa, ALT (12,4±2,6 UI/L), aspartato aminotransferasa, AST (48,1±9,3 UI/L), gammaglutamil transferasa, GGT (9,2±1,6 UI/L), lactato dehidrogenasa, LDH (117±22 UI/L), butiril colinesterasa, CHE

(168±32 UI/L) y creatin fosfoquinasa, CPK (432±85 UI/L). El crecimiento correlacionó con el aumento de peso (r =0,82, p =0,02). Se registró asociación lineal significativa entre el avance de la edad y la disminución de ALP (9 meses = 196 versus 21 meses = 102 UI/L), así como con los aumentos de CHE (126 versus 226 UI/L) y CPK (280 versus 572 UI/L). ALP y CPK resultaron más elevadas en machos que en hembras (p<0,05). Con excepción de GGT, las actividades enzimáticas fueron mayores en invierno que en las restantes estaciones del año. ALP, AST, GGT y LDH fueron significativamente más altas en el sistema de crianza donde el agua ocupó el 90% del piso de las piletas y el alimento se administró flotante, con relación a aquéllos donde el agua ocupó solo el 25% y alimento fue suministrado en el piso. Las enzimas indicadoras de sobrecarga hepática (ALP, ALT, AST, GGT y LDH) registraron los valores más altos en ranas alimentadas con vísceras (pulmón bovino, con y sin adición de pellets balanceados) y los más bajos en animales alimentados naturalmente en una laguna. Se destaca la utilidad del enzimograma para evaluar los estados metabólico, nutricional y sanitario, proponiéndose su aplicación como instrumento idóneo para optimizar la producción de carne de rana.

Palabras clave: *Rana catesbeiana*, enzimas plasmáticas, variaciones fisiológicas.

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