GLUCEMIA PHYSIOLOGICAL VARIATIONS OF GROWING BULL-FROG (*RANA CATESBEIANA*). ITS RELATIONSHIP WITH ALBUMINEMIA AND FRUCTOSAMINEMIA

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RESUMEN: Para obtener valores de referencia de glucosa plasmática en *Rana catesbeiana* en cautiverio, así como establecer variaciones fisiológicas atribuibles a sexo, edad, peso vivo, clima y sistemas de alimentación y manejo, se estudiaron 323 ejemplares sanos de ambos sexos y 9-21 meses de edad, mantenidos en 4 diferentes criaderos del nordeste argentino. Se obtuvo un intervalo de referencia de 2,75 ± 0,66 mmol/l. Los niveles de glucosa registraron variaciones significativas debidas a edad, peso vivo, clima, alimentación y manejo. La glucemia disminuyó ante el avance de edad y peso vivo ($p < 0,05$). Los más altos valores se obtuvieron en ranas alimentadas naturalmente en una laguna (3,13 mmol/l), y los más bajos en anfibios mantenidos con pulmón bovino molido (2,14 mmol/l), siendo intermedios en las dietas restantes (pellets balanceados). La glucemia fue más baja en invierno (hibernación) que en el resto de las temporadas del año (2,15 versus 3,41 mmol/l). No se encontraron diferencias significativas entre sexos, ni tampoco asociaciones lineales significativas entre glucosa, fructosamina, albúmina y peso vivo. En animales enfermos se hallaron valores de glucosa considerablemente alejados del intervalo de referencia. Se resalta la utilidad de la glucosa plasmática para evaluar el estado sanitario, metabólico y nutricional de las ranas productoras de carne.

ABSTRACT: The purpose of this study was to determine plasmatic glucose reference values and sex, age, liveweight, climate, and breeding and feeding systems physiological variations in captive bullfrog, *Rana catesbeiana*. Three hundred and twenty-three healthy animals (both sexes and 9 to 21 months old) reared in 4 different hatcheries from Corrientes, northeastern Argentina, were studied. A 2.75 ± 0.66 mmol/l glucose reference interval was obtained, and significant physiological variations due to age, liveweight, climate, feeding and handling system, were registered. Glucemia decreased according to both age and liveweight developments ($p<0.05$). Highest glucose values occurred in frogs fed naturally in a lagoon (3.13 mmol/l), while the lowest in amphibians maintained on bovine milled lung (2.14 mmol/l), being intermediates in remaining diets (balanced pellets). In winter (hibernation) frogs registered significantly lower glucose levels than in the remaining seasons (2.15 versus 3.41 mmol/l). No significant differences were found between sexes, as well as no significant lineal association among fructosamine, albumin and liveweight. It was found that glucose values largely distanced from the reference interval in sick animals. Utility of plasmatic glucose to evaluate sanitary, metabolic, and nutritional state in meat production frogs, is emphasized.

Palabras claves: *Rana catesbeiana*, glucemia, fructosaminemia, albuminemia, edad, sexo, peso, clima, alimentación, manejo de criadero

Key words: *Rana catesbeiana*, glucemia, fructosaminemia, albuminemia, age, sex, liveweight, climate, feeding, hatchery handling system

INTRODUCTION

Rana catesbeiana (Shaw, 1802) is an amphibian which meat is eatable, well-regarded because it has scarce fat and cholesterol proportion (Lima and Agostinho, 1992). In Argentina, there are more than 200 bullfrog hatcheries which produce meat

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marketed at a high price; captive *R. catesbeiana* is generally fed with balanced pellets which are similar to those elaborated for fish, as its nutritional requirements are still unknown (Carnevia, 1995). Knowledge of biochemical nutritional indicators, as glucemia, could contribute to establish the true nutritional requirements for this amphibian.

Amphibian blood values and their physiological variations due to age, sex, liveweight, food type, handling system, and season (climate) are yet to be studied. Plasmatic composition would be influenced by particular 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 (Goldstein, 1982; Rocha and Branco, 1998; Bicego and Branco, 1999; Busk *et al*., 2000; Coppo, 2001a; Winmill *et al*., 2005).

On physiological conditions, both ectothermal and endothermal animals principally obtain energy from glucose and fatty acids. Such energy sources come from hepatic or muscular glucogen respectively, as well as from hepatic or subcutaneous lipids (Kaneko, 1989). Frogs also store triglycerides as periovarian panniculus (Lima and Agostinho, 1992).

Glucemia is a nutritional indicator that rises in post-prandial state, and falls during fast and malnutrition; pathologically, it increases when insulin secretion decreases (Kaneko, 1989). Frogs glucemia is hormonally regulated. Amphibians pancreatic beta cells segregate insulin in a mammals mechanism similar way: they recognize metabolizable carbohydrates as glucose, mannose and fructose, but not other non-metabolizable as galactose and 2-desoxyglucose; insulin release is influenced by environmental temperature, increasing below 37ºC, and *vice versa* (Francini and Gagliardino, 1997).

Physiologically, insulin promotes the entrance of circulating glucose into cells, and post-prandial hyperglucemia correction. On the contrary, as defensive mechanism, other hormones (catecholamines, cortisol, glucagon) cause increase of glucemia, for example during sympathetic alarms or stress (Coppo, 2002).

Glucose can link to several circulating proteins, as hemoglobin and albumin. It is a non enzymatic process, which magnitude depends on prolonged permanency of high or low blood glucose levels (Coppo and Mussart, 1997). Prolonged hyperglucemia causes high glycosilation rate, and *vice versa*. Protein-glucose linkage generates "glycosilated proteins", as glycohemoglobin and fructosamine. The latter is an glycosilated albumin, and it is useful for a retrospective evaluation of the hydrocarbonated metabolism state. Persistent hypo- and hyperglucemia cause decrease and increase of fructosaminemia respectively, but transitory plasmatic glucose changes are not able to alter fructosamine level (Coppo, 2001b). In human beings, there was no significant difference in serum fructosamine levels when measured fasting and 2 hours after ingestion of 7 grams of glucose (Peiris, 2000).

Albumin is and effective nutritional indicator of protein intake and hepatic biosynthesis (Kaneko, 1989). Albuminemia decrease (malnutrition, hepatopathy, proteinuric nephropathy) causes fructosaminemia decrease. Low fructosamine results may be seen with decreased serum albumin, increased protein loss, or a change in the type of protein produced by the body (Coppo, 2001b). Reports about correlation between plasmatic fructosamine, albumin and glucose *R. catesbeiana* levels, were not found.

The purpose of this study was to obtain serum glucose reference values and to determine eventual age, sex, liveweight, food, year season (climate) and rearing system (handling) attributable physiological variations, as well as to verify possible lineal associations between glucemia, fructosaminemia and albuminemia in growing *R. catesbeiana* reared in hatcheries from north east of Argentina.

MATERIALS AND METHODS

Experimental subjects, feeding and handling. For a period of three years, 323 healthy *R. catesbeiana* specimens were used. Two hundred and seventy were maintained in intensive systems, located in 3 different 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. Other 32 animals were reared in an extensive system (semi-captivity), in a closed lagoon where frogs exclusively selected "natural" food (diet 2). They were adults, 16-20 month-old from both sexes. Thirty six per cent of the samples was taken during winter time, and 64% during the remaining seasons. Samples from sick animals were also taken; their values were not included for the reference interval calculation.

At breeding place #1 (Oberá, Misiones, north-east of Argentina), water came from natural slopes and it occupied 25% of tanks surface; food (diet 1) consisted in commercial balanced pellets for fish (45% protein), and it was supplied "dry" (scattered on the floor), sporadically accompanied by worms. Water from hatchery #2 (Paso de la Patria, Corrientes) came from an aquifer, extracted by means of a perforation; it covered 50% of tanks floor, and frogs were fed balanced pellets floating on the water (38% protein), occasionally supplemented by flies larva (diet 5). Hatchery #3 (Jardín América, Misiones, north-east of Argentina) also had gushing out water, which occupied 90% of the tanks; food was supplied floating on the water. During the first year, amphibians ate a mixture of equal parts of bovine milled lung and balanced pellets with 45% protein (diet 4), and during the second year lung was administered as unique food (diet 3). No heating system during winter season was used in the hatcheries; food was administered at a rate of 3-5% liveweight/day in all the cases.

Separately, samples from 21 *R. catesbeiana* healthy young specimens (50% each sex, 77 to 118 g liveweight), from a hatchery (# 4) located in Gran Guardia (Formosa, northeastern Argentina), were taken. Frogs were fed on floating balanced pellets (38% protein), bovine milled lung and tadpoles. These samples, coming from animals in active growing process (11-12 months of age), were used to verify lineal associations between glucose, fructosamine, albumin and liveweight.

Sample taking and laboratory procedures. Frogs were transported to the laboratory in thermal boxes which contained a 0.6% NaCl isotonic ice-cooled solution (2-3ºC); this procedure causes desensitization and lethargy, facilitating animal's manipulation (Lima and Agostinho, 1992). Liveweight was obtained in a Scientech-SL electronic balance, with an accuracy of 0.01 g. 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. Sample consisted in a venous and arterial blood mixture,

since frogs, with their anatomical characteristic, possess a unique ventricle (Goldstein, 1982). The blood was centrifuged (700g, 10 min) to obtain serum.

Chemical tests were carried out in a Labora Mannheim 4010 UV-visible spectrophotometer, using Wiener Lab reagents, by means of regular laboratory methods (Pesce and Kaplan, 1990): glucose (oxydase-peroxydase, measured at 505 nm), fructosamine (nitroblue tetrazolium, 530 nm), and albumin (bromide-cresol-sulphophthalein, 625 nm).

Experimental design and statistical analysis. A completely randomized design was used. Independent variables were age, sex, liveweight, year season (climate), and feeding and handling system (according to hatchery). Dependent variables (quantitative continuous) were glucose, fructosamine and albumin. The normality of the distribution was assessed using the Wilk-Shapiro test (WS). Parametric descriptive statistics included measures of central tendency (arithmetic mean, ξ), dispersion (standard deviation, SD) and ranges. Fiduciary probability was estimated by confidence intervals (CI±95%). After verifying homogeneity of variance (Bartlett test), the analysis of the variance (ANOVA) was calculated by one way lineal model. Following to the ANOVA, means comparison was carried out 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 this percentage the equality null hypothesis was rejected.

RESULTS AND DISCUSSION

General values obtained for plasmatic glucose (Table 1), showed an approximately normal distribution, which allowed the use of parametric statistics. Confidence intervals were adjusted around arithmetic means, but individual ranges were wide. Scarce regulation mechanisms and higher tolerance to hemodilution and hemoconcentration, would cause a great oscillation of blood values in frogs (Goldstein, 1982); the latter could explain the wide extent of glucose ranges obtained in this trial.

Table 1: Values obtained for the total studied population (n = 302).

	$\bar{x}$ ± SD	WS	CI ± 95%	range
Glucose (mmol/l)	2.75 ± 0.66	0.982	2.47 – 2.97	0.55 – 5.39

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

Comparatively, frog blood values were lower than those reported for human beings and carnivores; they were similar to those published for ruminants (Kaneko, 1989; Pesce and Kaplan, 1990; Sodikoff, 1996; Coppo, 2001a). After food ingestion, changes in amphibian plasma composition would be registered (Busk *et al*., 2000); other changes would also occur as a consequence of the circadian rhythm, caused by cortisol fluctuations (Coppo, 2001a). Both postprandial and circadian effects were ex-

cluded from the present study design, due to previous fast and basal condition of samples, and also because blood extraction was carried out in standardized morning hours.

Table 2: Differences according to age of amphibians ($\overline{x}$).

age (months)	glucose (mmol/l)
9	3.68[a]
10	3.52[a]
11	2.75[b]
12	3.02[ab]
13	2.81[b]
14	2.69[b]
15	2.58[b]
16	2.64[b]
18	2.64[b]
19	2.53[b]
20	2.20[bc]
21	2.31[bc]

In each column, different letters indicate significant differences (Tukey test, $p < 0.05$).

Glucose values gradually decreased with animals develop (Table 2), in opposition to the changes that happen in human beings and some domestic animals, in which highest glucemia is registered in the elderly (Coppo *et al*., 1998; Coppo and Mussart, 2000; Coppo, 2001a).

Table 3: Differences according to sex and liveweight of amphibians ($\overline{x}$).

	sex		liveweight (g)					
glucose	male	female	50-99	100-149	150-199	200-249	250-299	300-349
(mmol/l)	2.80[a]	2.69[a]	3.41[a]	3.63[a]	2.97[ab]	2.69[ab]	1.92[b]	2.03[b]

$\overline{x}$: arithmetic mean. In each file, different letters indicate significant differences (Tukey test, $p < 0.05$).

Table 3 reveals that, although male glucose levels were higher than those obtained on females, differences were not statistically significant. In human beings, is habitual that men plasmatic glucose concentration results higher than women, due to hormonal actions (Coppo and Mussart, 2000). Liveweight increase significantly correlated to glucose decrease. This change should be attributed to ontogenic reasons, because age and liveweight simultaneously increased, with a high degree linear association ($r = 0.82$, $p = 0.02$).

Table 4: Correlations registered among glucemia, age and liveweight.

	age (months)			liveweight (g)		
glucose	r	p	tendency	r	p	tendency
(mmol/l)	-0.87	0.0002	↓	-0.93	0.005	↓

r: correlation (Pearson test), p: significance.

Table 4 shows other significant correlations verified among glucose, age and liveweight. The monosaccharide always revealed lineal associations of negative sign (glucose decreased in opposition to both liveweight and age increases).

Table 5: Differences according to feeding type and hatchery handling system ($\overline{x}$).

	type of feeding					hatchery		
glucose	1	2	3	4	5	# 1	# 2	# 3
(mmol/l)	2.97^{a}	3.13^{a}	2.14^{b}	2.64^{ab}	2.80^{ab}	3.08^{a}	2.91^{a}	2.31^{b}

$\overline{x}$: arithmetic mean, 1: balanced, 2: natural, 3: milled lung, 4: balanced + milled lung, 5: balanced + flies larvas.
In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

Significantly lower glucose values were registered in frogs fed on viscera (bovine milled lung) and viscera + balanced pellets (Table 5). Highest glucose values happened when animals freely chose their food in the lagoon. In these frogs, necropsies allowed to verify that alimentary tract contained small fish, other frogs and tadpoles, crabs, and aquatic myriapods, coleopterons and hemipterans, as well as abundant grass.

Keeping in mind that glucemia is a nutritional indicator which varies according to food intake (Coppo, 2001a), the verified changes probably have been due to quality and/or quantity of supplied food. Authors demonstrated that growth of *R. catesbeiana* is markedly conditioned to food availability (Lima and Agostinho, 1992). According to hatchery handling system, frog glucose levels were significantly lower in hatchery # 3, where food was administered floating on the water.

Table 6. Differences according to climate ($\overline{x}$).

	season	
glucose	winter	rest of the year
(mmol/l)	2.15^{a}	3.41^{b}

$\overline{x}$: arithmetic mean. In each line, different letters indicate significant differences (Tukey test, $p < 0.05$).

During winter, frogs registered significantly lower glucose values, compared to the remaining seasons (Table 6). This fact would be due to low environmental temperature, which causes frog lethargy (hibernation) and feeding ceasing (Lima and Agostinho, 1992), the latter being in agreement to winter glucemia decreases verified in other investigations (Hill, 1980; Rocha and Branco, 1998; Bicego and Branco, 1999). Frogs metabolic rate would be regulated according to environmental temperature (Murata and Yamauchi, 2005), which variations would cause hormonal changes (Wright *et al*., 1999). Low-temperature arrest triiodothyronine-induced *R. catesbeiana* metamorphosis (Murata and Yamauchi, 2005). Temperature descent would cause insulin release and glucemia decrease in amphibians (Francini and Gagliardino, 1997).

Also, cold climate would cause amphibian cardiorespiratory regulation responses (Rocha and Branco, 1998; Bicego and Branco, 1999). Blockade of glycolysis during hypoxia (anaerobic metabolism) significantly reduced the time respiratory activity in *R.*

catesbeiana (Winmill *et al*., 2005). Hypoxia elicits a number of compensatory responses in animals, including behavioral hypothermia. Hypothesis that hypoglycemia induces hypothermia in *R. catesbeiana* was tested, this behavioral response would be beneficial (Rocha and Branco, 1998).

In their natural environment, *R. catesbeiana* young specimens would increase glucogen deposits before hibernation period, with decrease of both lipid deposits and plasmatic glucose levels (Farrar and Dupre, 1983). In other studies, blood glucose concentration increased from 2.21 ± 0,39 to 7.25 ± 1.14 mmol/l ($p < 0.01$) when frogs were transferred from 20 to –2ºC. Glucose accumulation in response to cold exposure was accompanied by a decrease ($p < 0.05$) of liver glycogen content, indicating that liver carbohydrate reserves were probably the primary carbon source of glucose synthesis, whereas muscle carbohydrate seems unimportant (Steiner *et al*., 2000). In the same way, glucemia winter descent registered in frogs of the present study should probably be attributed to hepatic glycogen depletion.

This phenomenon is common in fish, in which fast consumes glycogen and lipid hepatic deposits, and produces plasmatic glucose, triglycerides and protein decrease, as well as increase of circulating amino acids and fatty acids (Shimeno *et al*., 1990; Soengas *et al*., 1998). Concomitant enzymatic changes indicate hepatic glyconeogenesis activation (Tranulis *et al*., 1991), which uses amino acids to synthesize glucose (Sánchez Muros *et al*., 1998). This biosynthesis consumes muscular proteins and causes growth decrease and weight loss (Machado *et al*., 1988). In coincidence, *R. catesbeiana* also suffers considerable weight loss during hibernation (Coppo *et al*., 2004).

Table 7: Values obtained in frogs from hatchery # 4 (n = 21).

parameter	$\overline{x}$ ± SD	WS	CI±95%	range
glucose (mmol/l)	2.75 ± 0.88	0.956	1.92 – 3.63	0.77 – 5.11
fructosamine (umol/l)	141 ± 45	0.896	119 – 162	99 – 194
albumin (umol/l)	224 ± 32	0.964	206 – 243	167 – 278
liveweight (g)	91.6 ± 10.8	0.949	86 – 97	77 – 118

$\overline{x}$: arithmetic mean, SD: standard deviation, WS: Wilk-Shapiro distributive normality test (chart coefficient: 0.908, $\alpha = 0.05$), CI±95%: 95% confidence interval.

Table 7 shows values obtained in 21 young frogs (high growth rate). Age and feeding and handling system were homogeneous in these animals; samples were taken in summer. Liveweight and plasmatic glucose and albumin values were similar to those obtained in other hatcheries from frogs of the same age (Coppo *et al*., 2004). Reports on amphibian serum fructosamine values were not found. In frogs of the present study, mean fructosamine concentration was considerably lower than those obtained in adult dogs: 275±41 umol/l (Coppo and Mussart, 1997) and 2 months old calves: 297 umol/l (Coppo, 2001b).

There was not significant correlation between any couple of studied parameters. Keeping in mind the established significance ($p < 0.05$), the most approximate Pearson coefficient corresponded to lineal association between glucose and albumin ($r = 0.40$, $p = 0.07$). Absence of correlation between fructosamine and albumin could mean that

albumin variations (scarce) did not reach the magnitude necessary to significantly alter the glycosilation rate. In another study, it has been demonstrated that plasmatic albumin modifications should be considerable to be able to alter fructosamine level, in mammals (Coppo and Mussart, 1997).

Absence of correlation between glucose and fructosamine could imply that frogs glucose changes (very marked), should have been transitory: hyperglucemic peaks caused by sympathetic alarms or hypoglucemic episodes caused by previous fast, in coincidence to similar facts verified in mammals (Coppo, 2001b). In addition, authors affirm that pronounced variations (peaks) in the cortisol concentrations indicate that hormone levels are a function of the time of the day, and the environmental lighting regimen, which needs to be taken into account in measuring the effects of plasma hormones in amphibians (Wright *et al*., 2003).

Samples of frogs with presumptive health state deterioration were taken in several occasions, although such values were excluded from the statistics. Symptoms as adynamia, weakness, anorexy, dehydration, weight loss and skin abnormal coloration, were related with individual glucose values extremely distanced from the reference interval (0.02 to 11.0 mmol/l). Alterations verified in sick animals suggest that glucemia could be an effective indicator of nutritional, sanitary and metabolic dysfunctions in amphibians, just as it occurs in other species (Kaneko, 1989; Pesce and Kaplan, 1990; Sodikoff, 1996; Coppo, 2001a).

In conclusion, glucemia reference interval for *R. catesbeiana* reared in hatcheries from northeastern Argentina is 2.75 ± 0.66 mmol/l, without sex significant variations. Glucemia decreases when age, liveweight and environmental cold increase, varying according to feeding and handling systems. Absence of significant correlations between glucose, albumin and fructosamine values, indicates that registered marked glucemia changes should have been transitory, because the indicator of retrospective hydrocarbonated metabolism was not affected.

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BIBLIOGRAPHY

BICEGO, K.C. and L.G. BRANCO, 1999. Seasonal changes in the cardiorespiratory responses to hypercarbia and temperature in the bullfrog, *Rana catesbeiana*. *Comp. Biochem. Physiol.*, 124: 221-229.

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 VIII Jornadas Veterinarias de Corrientes*, Argentina: 81.

COPPO, J.A. and N.B. MUSSART, 1997. Serum fructosamine: a reference interval for a heterogeneous canine population. *Vet. Res. Comm*., 21: 471-476.

COPPO, J.A.; N.B. MUSSART; J.J. LÓPEZ and L. JOVANOVICS, 1998. Modificaciones de los analitos sanguíneos en el perro de edad avanzada. *Selecc. Vet.*, 6: 540-544.

COPPO, J.A. and N.B. MUSSART, 2000. Evolución de valores de la bioquímica clínica conforme avanza el envejecimiento. Estudio en población del nordeste argentino. *Prensa Méd. Arg.*, 87: 717-727.

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

Coppo, J.A., 2001b. Evolution of fructosaminaemia and glucaemia during the growth of unweaned and early weaned half-bred zebu calves. *Vet. Res. Comm.*, 25: 449-459.

Coppo, J.A., 2002. ¿Estrés o alarma simpática? Actualización bioquímico-clínica. *Selecc. Vet.*, 9: 336-342.

COPPO, J.A.; N.B. MUSSART and S.A. FIORANELLI, 2004. Impacto de la alimentación, clima y sistema de manejo del criadero sobre el peso y las proteínas plasmáticas de la rana toro, *Rana catesbeiana*. *Vet. Arg.*, 21: 101-116.

FARRAR, E.S. and R.K. DUPRE, 1983. The role of diet in glycogen storage by juvenile bullfrogs prior to overwintering. *Comp. Biochem. Physiol.*, 75: 255-260.

FRANCINI, F. and J.J. GAGLIARDINO, 1997. Efecto de la temperatura sobre la secreción de insulina *in vitro* en *Bufo arenarum*. *Anales III Congreso Argentino de Herpetología*, Corrientes, Argentina: 38.

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

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

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

LIMA, S.L. and C.A. AGOSTINHO, 1992. *A Tecnología de Criaçao de Rás*. Imprenta Universitaria, Vicosa. 288 p.

MACHADO, C.R.; M.A. Garófalo; J.E. Roselino; I.C. KETTELHUT and R.H. MIGLIORINI, 1988. Effects of starvation, refeeding, and insulin on energy-linked metabolic processes in catfish (*Rhamdia hilarii*) adapted to a carbohydrate-rich diet. *Gen. Comp. Endocrinol.*, 71: 429-437.

MURATA, T. and K. YAMAUCHI, 2005. Low-temperature arrest of the triiodothyronine-dependent transcription in *Rana catesbeiana* red blood cells. *Endocrinology*, 146: 256-264.

PEIRIS, D.S., 2000. The value of serum fructosamine in comparison with oral glucose tolerance test as a screening test for detection of gestational diabetes mellitus. *J. Obstet. Gynaecol.*, 20:136-138.

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

ROCHA, P.L. and L.G. BRANCO, 1998. Physiological significance of behavioral hypothermia in hypoglycemic frogs (*Rana catesbeiana*). *Comp. Biochem. Physiol.*, 119: 957-961.

SÁNCHEZ MUROS, M.J.; L. GARCÍA REJÓN; L. GARCÍA SALGUERO; M. de la HIGUERA and J. LUPIÁÑEZ, 1998. Long-term nutritional effects on the primary liver and kidney metabolism in rainbow trout. Adaptative response to starvation and a high-protein, carbohydrate-free diet on glutamate dehydrogenase and alanine aminotransferase kinetics. *Int. J. Biochem. Cell Biol.*, 30: 55-63.

SHIMENO, S.; D. KHEYYALI and M. TAKEDA, 1990. Metabolic adaptation to prolonged starvation in carp. *Nipp. Suisan Gakkaishi*, 56: 35-41.

SODIKOFF, C.H., 1996. *Laboratory Profiles of Small Animal Diseases*. 2nd ed. Mosby, Baltimore. 435 p.

SOENGAS, J.L.; E.F. STRONG and M.D. ANDRES, 1998. Glucose, lactate and beta-hydroxybutyrate utilization by rainbow trout brain: changes during food deprivation. *Physiol. Zool.*, 71: 285-293.

STEINER, A.A.; S.O. PETENUSCI; L.G. BRENTEGANI and L.G. BRANCO, 2000. The importance of glucose for the freezing tolerance/intolerance of the anuran amphibians *Rana catesbeiana* and *Bufo paracnemis*. *Rev. Bras. Biol.*, 60: 321-328.

TRANULIS, M.A.; B. CHRISTOPHERSEN; A.K. BLOM and B. BORREBAEK, 1991. Glucose dehydrogenase, glucose-6-phosphate dehydrogenase and hexokinase in liver of rainbow trout (*Salmo gairdneri*). Effects of starvation and temperature variations. *Comp. Biochem. Physiol.*, 99: 697-691.

WINMILL, R.E.; A.K. CHEN and M.S. HEDRICK, 2005. Development of the respiratory response to hypoxia in the isolated brainstem of the bullfrog *Rana catesbeiana*. *J. Exp. Biol.*, 208: 213-222.

WRIGHT, M.L.; K.L. PROCTOR and C.D. ALVES, 1999. Hormonal profiles correlated with season, cold, and starvation in *Rana catesbeiana*. *Comp. Biochem. Physiol.*, 124: 109-116.

WRIGHT, M.L.; C.J. GUERTIN; J.L. DUFFY; M.C. SZATKOWSKI; R.F. VISCONTI and C.D. ALVES, 2003. Developmental and diel profiles of plasma corticosteroids in the bullfrog, *Rana catesbeiana*. *Comp. Biochem. Physiol.*, 135: 585-595.

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