

Malnutrition in farm-cultured bullfrog (*Rana catesbeiana*) fed on bovine milled lung in Argentina



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Abstract

The true nutritional requirements of bullfrog (*Rana catesbeiana*, Anura: Ranidae) are still controversial. In Argentine hatcheries, bullfrogs are fed on commercial balanced diets elaborated for fish; high price of balanced food decreases the relationship cost / benefit. This study had the objective to verify the nutritious effectiveness of bovine lung, by means of liveweight gains and metabolic and nutritional biochemical indicators controls. Eighty frogs were randomly divided into experimental (E) and control (C) groups of 40 animals each. Controls consumed a fish commercial balanced diet (23% protein in dry matter, DM), while those in group E were fed on bovine milled lung (16% protein, DM). Food was administered at a rate of 5% of liveweight/day (DM) for both groups. The weighings and collection of samples were made on days 0 and 120. One frog of group C and 8 frogs of group E died during the study. Control animals did not register physical abnormalities. On the other hand, emaciation, adynamia, weakness, anorexy, and skin abnormal coloration were verified in some amphibians of group E. At the end of the study, liveweight and albumin, creatinine, urea, cholesterol, triglycerides, glucose, phosphorous and magnesium serum values, were significantly lower in group E than in group C ($p < 0.05$). This changes are attributed to metabolic imbalances related to malnutrition and reveal that bovine lung is not an appropriate food for captive bullfrog.

Key words: captive bullfrog, liveweight, malnutrition, serum biochemical indicators

Introduction

Northeastern Argentina is a privileged zone to rear bullfrogs (*Rana catesbeiana*, Shaw 1802), due to its subtropical climate. Its meat is eatable and well-regarded because it has scarce fat and cholesterol proportions (Coppo 2004). In natural environments, bullfrog has a voracious appetite and eats almost anything that moves and that it can swallow, including invertebrates and small vertebrates such as mammals, birds, reptiles, fish, even turtles and other frogs and tadpoles (Lima and Agostinho 1992).

Captive bullfrog fed on mice or rats revealed changes in metabolism and acid-base and respiratory parameters (Busk et al 2000). Properties of certain amphibian digestive enzymes, such as size, active site, profile of autolytic activation, as well as pH dependency, differ significantly from other vertebrate enzymes (Ikuzawa et al 2001).

In hatcheries, amphibians are fed on commercial balanced diets elaborated for fish, because their true nutritional requirements are still controversial (Hayashi et al 2004). This food should preferably float on the water. High price of balanced food decreases the relationship cost / benefit.

Bovine lung is a low cost viscera, that also floats on the water; for this reason, it was convenient to test its nutritious effectiveness in the final stage (fattening) of the rearing process, by means of liveweight gains and metabolic and nutritional biochemical indicators controls.

Materials and Methods

The study was carried out in summer, in a farm located in Corrientes, northeastern Argentina. One hundred frogs (3 months old and 80 g liveweight), 50% females and 50% males, clinically healthy and phenotypically homogeneous, were used.

Twenty animals were sacrificed at the beginning of the assay (day 0), to obtain initial blood serum values. The remaining frogs were randomly divided into experimental (E) and control (C) groups of 40 animals each, which stayed in contiguous tanks, submitted to similar management. Controls consumed a fish commercial balanced diet (23% crude protein, 3% ether extract, DM), while those in group E were fed on bovine milled lung (16% crude protein, 2% ether extract, DM). Food was administered at a rate of 5% of liveweight/day for both groups.

The weighings and collection of samples were made on days 0 and 120. Blood samples were taken by cardiac puncture, at 7-8 h each morning. The assays were performed at 37°C in a Labora Mannheim 4010 photometer (Labortechnik, Mannheim, Germany). The concentrations of serum albumin (bromide-cresol-sulphophthalein method, 625 nm), creatinine (alkaline picrate technique, 520 nm), urea (urease, 546 nm), total cholesterol (oxidase-peroxidase, 505 nm), triglycerides (lipase-peroxydase, 546 nm), glucose (oxidase-peroxidase, 505 nm), calcium (cresolphthalein complexone, 570 nm), inorganic phosphorous (pyridyl bisphenyl triazine sulphonate, 560 nm) and magnesium (xylydyl blue, 510 nm) were measured (Pesce and Kaplan 1990) using Wiener reagents (Riobamba 2944, Rosario, Argentina).

The normality of the distribution of the obtained values was assessed using the Wilk-Shapiro test. Parametric descriptive statistics (mean \bar{x} , standard deviation SD, and confidence interval $CI \pm 95\%$) were obtained by conventional procedures. Analysis of the variance (ANOVA) was calculated by one way linear model. All the calculations were made using the software Statistica, Version 1999. Statistical significance in this paper refers to the 5% level.

Results

One frog of group C and 8 frogs of group E died during the study. Control animals did not register physical abnormalities. On the other hand, emaciation, adynamia, weakness, anorexy, and skin abnormal coloration were verified in some amphibians of group E.

Table 1 shows the initial (day 0) and final values obtained in groups C and E for each studied parameter. All the initial values were statistically homogeneous (CI 95%) and had a symmetrical distribution in the Wilk-Shapiro test. At the end of the study, liveweight and serum values -except calcium- were significantly lower in group E than in group C ($p < 0.05$).

Table 1. Evolution of the studied parameters ($\bar{x} \pm SD$) in the control (C) and experimental (E) groups

Parameter	Initial (day 0) n = 20	Final day (120)	
		C (n = 39)	E (n = 32)
Liveweight (g)	79.8 \pm 11.3	171.4 \pm 21.7 ^a	144.6 \pm 27.3 ^b
Albumin (g/dL)	1.38 \pm 0.25	1.80 \pm 0.33 ^a	1.57 \pm 0.29 ^b
Creatinine (umol/L)	31.5 \pm 5.7	44.7 \pm 7.0 ^a	36.2 \pm 6.6 ^b
Urea (mmol/L)	1.07 \pm 0.18	1.92 \pm 0.29 ^a	1.58 \pm 0.30 ^b
Total cholesterol (mmol/L)	1.97 \pm 0.33	1.41 \pm 0.26 ^a	1.25 \pm 0.20 ^b
Triglycerides (mmol/L)	0.70 \pm 0.08	0.41 \pm 0.06 ^a	0.26 \pm 0.03 ^b
Glucose (mmol/L)	3.38 \pm 0.59	2.52 \pm 0.41 ^a	2.07 \pm 0.38 ^b
Calcium (mmol/L)	2.42 \pm 0.44	2.12 \pm 0.36 ^a	2.02 \pm 0.34 ^a
Inorganic phosphorous (mmol/L)	3.52 \pm 0.63	1.87 \pm 0.34 ^a	1.19 \pm 0.21 ^b
Magnesium (mmol/L)	0.94 \pm 0.15	1.09 \pm 0.14 ^a	0.86 \pm 0.12 ^b

^{a-b} In each row, different letters indicate significant differences between the means ($p < 0.05$)

Discussion

The initial values of the studied parameters corresponded with the serum reference values for the species, age, season, and geographical area (Coppo 2004). After food ingestion, changes in amphibian plasma composition would be registered (Busk et al 2000); other changes would also occur as a consequence of circadian rhythm, caused by cortisol fluctuations (Wright et al 1999). 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 standardized morning hours.

In group C, increase of serum albumin, creatinine and urea, as well as decrease of total cholesterol, triglycerides, glucose, calcium, and inorganic phosphorous, should be attributed to ontogeny (Mussart et al 2004). On the contrary, decrease of all the parameter concentrations in group E should be attributed to metabolic imbalances related to malnutrition. Serum concentrations of several studied parameters are directly proportional to the amount intake, and the appropriate synthesis of others depends on the balance of the nutrients in the diet, being diminished in nutritional deficiencies (Slobodianik et al 1999). The main problem that affects growth of captive frogs is the nutritional privation, which conditions the appearance of transmissible illnesses (Lima and Agostinho 1992).

Undernourished fish also decrease their protein, lipid and glucose serum concentrations, due to depletion of hepatic reserves (Soengas et al 1998, Shimeno et al 1990). Hypoglycemia induces hepatic gluconeogenesis, which uses amino acids coming from muscular proteins, causing growth decrease and weight loss (Machado et al 1988).

Conclusion

Weight gain reduction and alteration of nutritional and metabolic indicators in E, reveal that bovine lung is not an appropriate food for captive bullfrog.

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