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Liver histopathological changes in breeding bullfrogs

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ABSTRACT. In order to complement the histopathological study of juveniles and tadpoles of the bullfrog, *Lithobathes catesbeianus*, fed commercial diet used by frog farms in Rio de Janeiro, containing 40% crude protein, we performed necropsy and histopathology of the liver to establish a relationship between the quality of crude protein in the diet and animal health. For this, it was used twenty breeding male bullfrogs, with average weight 591.30 g (± 91.90 g) and length 165.02 mm (± 14.22 mm), and ten females with average weight and length of 629.80 g (± 134.47 g) and 169.32 mm (± 21.82 mm). The liver histopathology showed hyperemia, high number of melanomacrophages and cytoplasmic rarefaction, probably due to protein deficiency and fatty liver degeneration and presence of inflammatory processes. These lesions indicate a degenerative nutritional process. These findings suggest that the animals were fed with proteins of low biological value, indicating poor quality of feed, undermining the sanity. The impairment of liver function by these injuries will lead to reduced availability of precursors of sex hormones, since the liver is important in the metabolism of the same, and reproductive performance of these animals may be impaired.

Keywords: aquaculture, animal nutrition, protein malnutrition, histopathology, frog culture.

Alterações histopatológicas do fígado de reprodutores de rã-touro

RESUMO. Em complementação ao estudo histopatológico de girinos e imagos da rã-touro, *Lithobates catesbeianus,* alimentados com ração comercial utilizada pelos ranários do Rio de Janeiro, contendo 40% de proteína bruta, realizou-se necropsia e histopatologia do fígado de reprodutores, procurando uma relação entre a qualidade de proteína bruta na ração e a sanidade do animal. Foram utilizados 20 reprodutores de rã-touro, dez machos com peso de 591,30 g (± 91,90 g) e comprimento médios de 165,02 mm (± 14,22 mm), e dez fêmeas com peso e comprimento médios de 629,80 g (± 134,47 g) e 169,32 mm (± 21,82 mm). Os resultados da histopatologia mostraram que o fígado dos reprodutores, tanto machos quanto fêmeas apresentavam hiperemia, grande número de melanomacrófagos e rarefação citoplasmática provavelmente por deficiência proteica e quadro de degeneração hepática gordurosa. Tais lesões indicam quadro degenerativo nutricional. Também presente processos inflamatórios focais. Estes achados sugerem uma alimentação com proteínas de baixo valor biológico, indicando má qualidade da ração, comprometendo sua sanidade. Pode-se inferir que o comprometimento das funções hepáticas, irá diminuir a disponibilidade de precursores dos hormônios sexuais, pois o fígado é importante na metabolização dos mesmos, e a performance reprodutiva destes animais poderá ser prejudicada.

Palavras-chave: aquicultura, nutrição animal, desnutrição proteica, histopatologia, ranicultura.

Introdution

The breeding management and planning are important steps in livestock. Researches on bullfrog reproduction (AGOSTINHO et al., 2011; CASTRO et al., 2012; DIAS et al., 2010) have verified the influence of several factors, including the climate, mainly temperature and photoperiod, both with strong relationship with the animal metabolism.

The liver is, per excellence, a key organ to understand the health and nutritional status of the animals. Changes arising from food handling or other failures in zootechnical and sanitary management, or even the widespread presence of infectious agents reflect on its structure and function (HIPOLITO et al, 2001, 2004).

Unfortunately, knowledge about normal and abnormal functions of amphibian liver is still very limited. There are no clinical procedures or tools for an accurate diagnosis or acceptable prognosis. Thus, most liver disorders are only known with a retrospective diagnosis or by autopsy (CRAWSHAW; WEINKLE, 2000).

Seixas-Filho et al. (2008a and b) conducted studies on the liver of bullfrog tadpoles fed with

commercial ration showing 32, 36, 45 and 55% of crude protein (CP) and found the organs spotted and straw-colored, for all levels of CP, and the histopathologic analysis revealed a thinning and degeneration of cell protein, intestine with colitis and flattened microvilli, spleen with lymphocyte hyperplasia, heart without any change, kidneys with glomerulonephritis and areas of tubulonephrosis; mononuclear gastritis, hyperplasia and hypertrophy of regional lymph nodes. In all organs, there was hemosiderin deposit.

Still Seixas-Filho et al. (2009) studying bullfrog juveniles, *Lithobates catesbeianus*, fed with commercial rations with three levels of crude protein (CP; 28; 36 and 45%) sought to establish a relationship between quality of crude protein and animal health status on the histopathological examination of the liver. The results showed that animals fed with different diets, regardless of the level of CP had lymphocytic hepatitis, colitis and flattening of microvilli, kidneys with tubulonephrosis areas and renal tubules calcification, myocarditis and dissociation of cardiac muscle fibers.

In the feeding of bullfrogs, the whole performance is based on the quantity and quality of the protein in the diet. Any change in liver function caused by food, lack of food or good-quality protein or most needed amino acids, general organ dysfunction caused by stress or damage suffered by infectious and parasitic agents that can gravely compromise the liver, will also cause impairment of the intricate metabolic organic relationship.

For example, the liver dysfunction causes impairment of proteins and amino acids metabolism, which interferes with the hormones production, such as corticotropin releasing factor (CRF) and glucocorticoids which are components of the hypothalamic-pituitary-adrenal (HPA) axis or stress axis and play an important role in central regulation of energy balance and food intake (RICHARD et al., 2002).

This study aimed to examine possible relationships between diet and health in adult breeding animals (*Lithobates catesbeianus* Shaw, 1802), assessed by the liver.

Material and methods

The experiment was conducted at the Biology Research Laboratory of the Augusto Motta University Center - Unisuam, Bonsucesso, *Campus* in Rio de Janeiro State and at the Biological Institute of São Paulo State.

Breeding bullfrogs (*Rana catesbeiana* Shaw 1802), today called *Lithobates catesbeianus* (FROST et al., 2006) were derived from UNISUAM experimental frog farm, reared in the semi-flooded system, kept in maintenance bays, separated by sex, fed twice a day with commercial feed containing 40% crude protein (Table 1).

Table 1. Assurance level and proximate composition of commercial diet used in the feeding of breeding bullfrogs.

	Assurance level	Analyzed composition[1]
Maximum moisture (%)	13.0	9.54
Crude protein (%)	40.0	41.85
Ether extract (minimum) (%)	10.0	6.79
Fibrous material (maximum) (%)	6.0	6.0
Ash (maximum) (%)	13.0	9.81
Calcium (maximum) (%)	2.5	2.5
Phosphorus – P (minimum) (%)	1.0	1.0
Crude energy (kcal kg^{-1})		4.501
Carbohydrates (%)		49.75

Basic Composition: Soybean meal, Fishmeal, Wheat bran, Corn gluten meal 60%, meat and bone flour, Corn, Blood meal, Fish oil, Calcium carbonate, Dicalcium phosphate, Salt vitamin supplement (1), Salt mineral supplement (2), Antioxidant (3). (1) Composition per kg: vitamin A, 12.000 UI; vitamin D3, 4.000 UI; vitamin E, 150 UI; vitamin K, 10 UI; Folic Acid, 10 mg; biotin, 0,8 mg; coline, 500 mg; niacin, 150 mg; calcium pantothenate, 50 mg; thiamine, 30 mg; riboflavin, 30 mg; pyridoxine B6, 30 mg; vitamin B12, 35 μg; vitamin C, 300 mg. (2) Composition per kg: Mg, 700 mg; Mn, 30 mg; Zn, 200 mg; Cu, 15 mg; Fe, 100 mg; I, 1 mg; Se, 0,3 mg. (3) Ethoxyquin, 250 mg.

To perform the experiment, twenty individuals were randomly collected; ten males and ten females, at 18 months of age.

Weight and length of the animals with their respective means and standard deviations are presented in Table 2.

Table 2. Weight and length of breeding bullfrogs used in the experiment.

	Male		Female	
Animal	Weight (g)	Length (mm)	Weight (g)	Length (mm)
1	419.0	150.34	776.0	210.98
2	560.0	156.43	643.0	196.52
3	741.0	183.97	609.0	159.54
4	621.0	156.93	395.0	150.50
5	507.0	153.98	468.0	150.90
6	532.0	159.40	675.0	153.99
7	648.0	195.32	763.0	163.63
8	678.0	169.20	505.0	154.02
9	618.0	163.41	772.0	190.96
10	589.0	161.18	692.0	162.21
Mean	591.3	165.02	629.8	169.32
Standard deviation	91.9	14.22	134.5	21.82

For the histopathological analysis, the animals were placed in containers with water at 0°C, in order to promote the reduction of the metabolism, and anesthetized with a solution of menthol, numbed by physiological restraint, by transverse section of the spinal cord; subjected to necropsy to verify the occurrence of external or internal injuries, and histopathological aspect, especially the liver. The organs were set in metacarmim solution (PUCHTLER et al., 1970), for 12 hours and then kept in 70% alcohol. For histopathological examinations, they were embedded in paraffin, cut to 5 mm thick on microtome and stained with HE (hematoxylin and eosin) for observation under light microscopy.

The animals received coding in histological slides to ease identification, namely: MR - Breeding Male and FR - Breeding Female.

Results and discussion

The experimental environment provided comfortable conditions to the animals throughout the observation period, as evidenced by the continuous feeding, and complete adaptation to the environment.

On histopathological examination of the liver, there was a high prevalence of protein-mineral degeneration in hepatocytes, regardless of the amount of protein in the feed given to the animals. The livers were macroscopically pale and microscopically with parenchyma composed of hepatocytes with markedly rarefied and vacuolated cytoplasm, resembling a lace (Figures 1, 2 and 3).

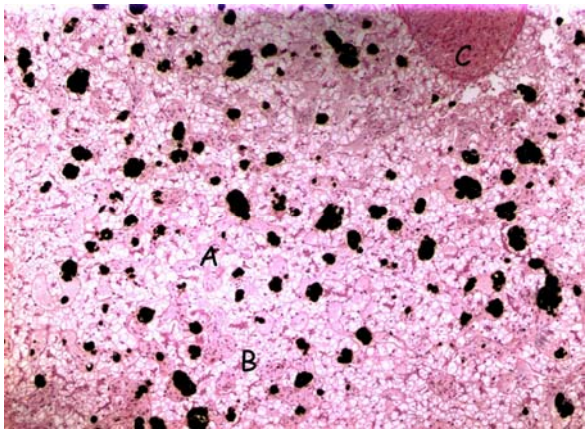

Figure 1. Photomicrography of adult bullfrog liver; reproductive male. Accented rarefaction of the hepatic cells cytoplasm (A) and presence of melanin deposit (B). Congested sinusoid vases (C). Staining by HE, 40X. (The same chart was observed in other reproductive males).

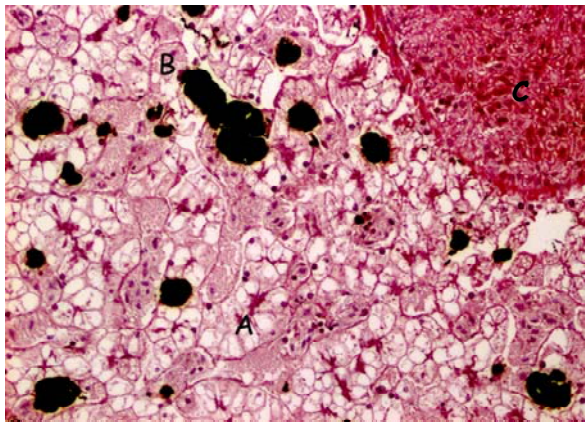

Figure 2. Photomicrography of adult bullfrog liver; reproductive male. Accented rarefaction of the hepatic cells' cytoplasm (A), deposit of melanin (B) and dilated vases (C). Staining by HE, 100X. (The same chart was observed in other reproductive males).

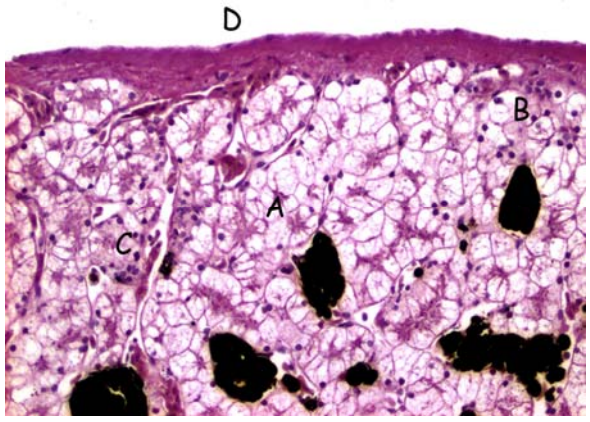

Figure 3. Photomicrography of adult bullfrog liver; reproductive female. Accented rarefaction of the hepatic cells' cytoplasm (A), presence of subcapsular hepatitis (B) and focal (C) and thickening of hepatic capsule (D). Staining by HE, 100X. (The same chart was observed in other reproductive females).

In the observations, 90% of the animals analyzed showed a lack of protein deposition in hepatocytes, suggesting that, the diets contained poor-quality protein.

These lesions suggested degeneration of nutritional origin, with development of inflammatory processes, spreading to all organ.

Probably the animals were fed with proteins of low biological value, indicating, apparently, the poor quality of rations, which compromises health and, consequently, performance, mainly, the reproduction.

The liver is responsible for the synthesis and metabolism of a key protein, the cholesterol. This is an essential part of the cell membrane and is required for the production of certain hormones, like estrogen, testosterone, adrenaline and noradrenaline. The liver also converts substances of the digested food into proteins, fats and

carbohydrates. Sugars are stored in the liver in the form of glycogen and, when necessary, when the concentration of blood sugar becomes too low, are broken down and released into the bloodstream in the form of glucose. Another liver function is the synthesis of many important compounds, especially proteins, which the body uses to perform different functions (GUYTON; HALL, 2006).

Proteins are considered the main constituent of any living cell and represent the most abundant chemical group of the animal body (OLVERA-NOVOA et al., 2007), with exception of water. Proteins are essential to the nucleus and cytoplasm and, summarizing, have the functions of repairing damaged or worn tissues and form new ones (synthesis of new proteins during growth). Proteins, via food, can be catabolized and act as energy source or as substrate for the formation of lipids and carbohydrates in the tissue, as well as hormones, enzymes, and a variety of important substances such as antibodies, hemoglobin, among others (TATTERSALL; ULTSH, 2008).

Once the liver is the "guardian of the internal environment", liver disorders have far-reaching effects on the body homeostasis. The prolonged protein-calorie malnutrition produces a wide range of syndromes in mammals, including the immune resistance deficiency by lymphoid atrophy and favoring the occurrence of infectious diseases.

These breeding animals live on average far more than animals slaughtered commercially for meat production. This longer life, under a condition of protein deficiency, can be reflected in a reduced organic defense, favoring inflammatory processes, as noted; however due to good breeding conditions, with constant feeding and hygiene, and shelter space, where external factors of stress have been minimized or eliminated, these animals can survive (HIPOLITO, 2003).

Considering that they are breeding animals and the impaired liver conditions, whereby the essential is the maintenance of life itself, surely the reproductive performance will be affected and may result in decrease in spermatogenesis and production of eggs.

The glucose control in frogs, as in mammals, has participation of the endocrine system. In vitro studies of the carbohydrate metabolism in isolated frog hepatocytes revealed that they are sensitive to insulin and glycogenolytic hormones, similar to verified in mammal hepatocytes. In this way, there was an increase in liver glycogen reserves concomitant with the elevation and depletion of insulin in the presence of glycogenolytic hormones (JANSSENS; GRIGG, 1987).

Facing the variation in plasma concentration of lipids, it has been suggested that they are used by muscles as a source of energy, forming a glucose-sparing system, directing it preferably to the maintenance of the nervous system homeostasis, as suggested in different countries with well-defined seasons (KING et al., 1995).

Furthermore, studies have assessed the seasonality of the glycogen phosphorylase and glycogen synthase enzymes in the green frog (*Rana esculenta*) liver and have found that phosphorylase is more active during wintertime, while the synthase, during the summer, suggesting a seasonal influence on the activity of these enzymes (SCAPIN; DI GIUSEPPE, 1994). Although there is little information about the enzymes involved on the glycogen metabolism in amphibians, it is assumed that it occurs similarly to other vertebrates and that it might suffer the influence of seasonality (PETERSEN; GLEESON, 2007, 2009, 2011).

In practical terms, for example in commercial rearing, this might not be noticed. The simultaneous raising of males and females in the same pond, and the non-individualized collection of eggs lead the rates of fertility and hatchability to be considered as normal within a zootechnical expected range.

To know exactly if the severe injuries observed in the liver significantly interfere on spermatogenesis and the production of roe, and thus in fertilization and hatching, the couples need to be separated and each collection of eggs monitored individually.

No reference was found dealing with this subject among amphibians, liver damage and reproductive indices, leading to a new path that should be explored in further studies.

Conclusion

These findings suggested that the animals were fed with proteins of low biological value, indicating poor quality of feed, compromising their sanity. The impairment in liver function by these injuries will probably lead to a reduced availability of precursors of sex hormones in these animals, once the liver is an important organ for the metabolism of the same, and the reproductive performance of these animals may be negatively affected.

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