ENZYMATIC ACTIVITY OF LIPASE IN POST-METAMORPHIC PHASE BULLFROGS

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ABSTRACT: The knowledge of the digestive system of bullfrogs is an important step for the determination of their nutritional requirements throughout growth phases. With the objective of evaluating the enzymatic activity of lipase in the intestinal content of bullfrogs (*Rana catesbeiana* Shaw, 1802), 100 animals with median weight of 3.6 g were distributed in stalls under controlled temperature and photoperiod. The frogs, selected at the post-metamorphic phase, received commercial extruded diet *ad libitum* throughout the 87-day experiment. The collections of the intestinal content were performed by the desensitization of the frogs in ice and water at 0ºC and subsequent isolation of the small intestine. Determination of lipase activity was performed with a commercial enzymatic kit (Lipase-Bioclin, MG, Brazil), first measured in samples taken at day three (3.46 UI). During the initial phase the bullfrog possesses low lipase hydrolysis capacity was found, having a specific activity of 217 UI mg^{-1}. In the subsequent period both lipase activity and specific lipase activity continuously increased. Lipase activity as a function of bullfrog weight fell after day twenty and reached 0.33 UI g^{-1}, for frogs of medium weight (179 g). Feed for bullfrogs at the post-metamorphic phase weighing more than 10 g can have larger amounts of ingredients containg lipids, due to the increased digestive capacity of these frogs.
Key words: digestive enzyme, frog culture, specific activity

ATIVIDADE ENZIMÁTICA DA LIPASE EM RÃ-TOURO NA FASE PÓS-METAMÓRFICA

RESUMO: O conhecimento do sistema digestório da rã-touro é um passo importante para a determinação de sua exigência nutricional nas diferentes fases de crescimento. Com o objetivo de avaliar a atividade enzimática da lipase no conteúdo intestinal da rã-touro (*Rana catesbeiana* Shaw, 1802), 100 animais com peso médio de 3,6 g foram distribuídos em baias-teste com temperatura e fotoperíodo controlados. As rãs, selecionadas na fase pos-metamórfica receberam ração extruzada comercial *ad libitum*. Durante 87 dias de experimento, 29 coletas (87 rãs) foram feitas em intervalos que variaram de um a oito dias. As rãs foram insensibilizadas em água e gelo a 0ºC para subseqüente isolamento do intestino delgado e retirada do conteúdo intestinal. Para determinação da atividade da lipase foram usados conjuntos enzimáticos da BIOCLIN. A atividade da lipase foi registrada no terceiro dia (3,46 UI). Na fase inicial, a rã-touro possui baixa capacidade de hidrólise para a lipase que teve atividade específica de 217 UI mg^{-1}. No período subseqüente, observou-se aumento contínuo da atividade e da atividade específica da lipase. A atividade da lipase em função do peso da rã-touro depois do 20º dia apresentou redução contínua, alcançando valor de 0,33 UI g^{-1}, registrado para rãs com peso médio de 179 g. As rações para rã-touro com peso superior a 10 g podem conter quantidades maiores de ingredientes que contenham lipídos, devido ao aumento da capacidade da digestão.
Palavras-chave: atividade específica, enzima digestiva, ranicultura

INTRODUCTION

According to FAO (2005) annual frog production in Brazil is of the order of 600 t, which represents 0.2% of the country's total aquaculture production. The annual world trade of frog meat reaches the order of two hundred tons. France and the United States are the main consumers and importers of frog meat. Asian countries are the largest suppliers of frogs to the world market. Frogs supplied to the international market are both raised in captivity, as well as captured in flooded rice fields. Captured wild frog stocks are demonstrating signs of decline in recent years (Lima et al., 1999).

With the recent development of the frog culture, new knowledge in the area of frog nutrition in the aquatic and post-metamorphic phases was acquired (Casali et al., 2005; Hayashi et al., 2004; Braga et al., 2004; Rodrigues et al., 2004; Pryor, 2003; Lima et al., 2003, Figueiredo et al., 2001). However, these studies lack standardization of methodologies to obtain consistent and comparable data. Little information can also be found in the literature in respect to nutrition and feeding of bullfrogs. This lack of information has impeded the formulation of feeds with nutrient contents compatible with their nutritional requirements during the different developmental phases.

The efficient use of ingredients in feeds is directly related to the digestive process, in which the enzymes have a fundamental function. The biochemical-enzymatic study of digestive enzymes becomes indispensable for the understanding of the physiology of the digestion and metabolism of nutrients. In higher vertebrates digestive enzymes are distributed all along the digestive tract. These enzymes are restricted to distinct gut sections, showing a clear functional zonation. This arrangement has not been clearly observed for most fish, for which they are usually well distributed throughout the tract. This type of information assists nutrition studies and makes more accurate diet formulations possible (Lundstedt et al., 2004).

Borlongan (1990) reported that lipase is widely distributed in the digestive tract of "milkfish" (*Chanos chanos*), however, its activity is greater in intestinal extracts, with the upper portion having larger activity than the lower portion. This author detected significant activity of lipase in extracts of the esophagus. This indicates a more active function of this organ in the digestive process. In contrast, for the Nile tilapia, the most intense lipase activity was present in the cranial portion of the intestine (Tengjaroenkul et al., 2000).

Information regarding the study of the enzymatic activity in amphibians is scarce in the literature. Farrar & Dupre (1983) verified that during the pre-winter period, for *Rana catesbeiana* consuming food, the digestive enzymes are liberated for digestion. This guarantees that the carbohydrate and lipid supplies are used for energy reserves.

The study described here was designed to evaluate the enzymatic activity of lipase in the small intestine of the bullfrog (*Rana catesbeiana* Shaw, 1802) when weighing 3.6 to 200 g.

MATERIAL AND METHODS

An experiment with *Rana catesbeiana* in the tadpole phase, was carried out Viçosa, MG, Brazil (20º28' S and 42º20' W). At the end of the metamorphosis process juvenile frogs (imagos) were transferred to the raising section. There a screening was made for weight uniformity. Each group of 25 animals with medium weight of 3.6 g was housed in four suspended test stalls, in controlled temperature (27±1.0°C) and photoperiod (12 hours) rooms.

Stalls were cleaned, water exchanged and the feeding of the animals made once daily throughout the test period. A commercial extruded feed with 42% crude protein, 4533 kcal kg^{-1} gross energy and 9.72% crude fat, was supplied *ad libitum* in feeders together with *Musca domestica* larvae (used to condition frogs to the feed). The amount of larvae initially consisted of 30% of the feed and was reduced to 5% gradually during the first 30 days.

For lipase activity evaluation, three frogs were collected in intervals established in order to obtain groups of animals in different weight categories during the 87 days of the experiment. During the first ten days, samples of the contents of the small intestine were made daily. During the remaining period (77 days), nineteen additional samples were analyzed in larger intervals, i.e., five collections were made for each interval of two, three and four days, respectively, and four collections were made with intervals of eight days. The last collection corresponded to the first day a frog of 200 g was collected.

Three hours after feeding frogs were tagged, weighed and desensitized in water and ice at 0°C Immediately after desensitization the small intestine was removed using forceps and scissors and the contents wrapped in aluminum foil, then immersed in liquid nitrogen and thereafter maintained at -40°C. Samples were freeze-dried and stored at -20°C before lipase activity analysis. In this process, eighty seven bullfrogs were sacrificed.

Chyme samples were prepared first taking freeze-dried material (1 mg) dissolved in 0.5 ml distilled water in plastic tubes. Next, the solution was centrifuged at 35,000 × g, 4°C for 20 minutes. Finally, an aliquot of the supernatant was taken for enzymatic activity determination.

Lipase acts hydrolyzing the links of glycerol esters and fatty acids of long chains producing diacylglycerols, monoacylglycerols and free fatty acids. During the reaction the substratum, in buffered and stabilized medium, acquires an emulsified form (micelles) with lipid-water interfaces necessary for lipase action. In the presence of the dithionitrobenzoic acid, lipase activity develops with a yellow coloration with color intensity proportional to the enzyme concentration (Cherry & Crandall, 1932). For activity analysis the Lipase Kit (Bioclin, MG, Brazil) was used. Samples were measured with a BECKMAN DU-70

spectrophotometer at an absorbance wave length of 410 nm. Lipase activity was calculated according to:

$$\text{Lipase (UI)} = \frac{\text{Abs (Sample)} - \text{Abs (Control)}}{7} \text{x1000}$$

where: Abs (Sample) = absorbance of the sample; Abs (Control) = absorbance of the control.

The determination of the protein concentration was carried out by absorption readings of samples at 260 and 280 nm, following the method described by Warburg & Christin (1941). To obtain specific activities of the digestive enzymes in the chyme, the activity of the enzyme was divided by the protein concentration, both obtained on the same sample. To obtain the activity of the lipase in relation to the weight of the animals the activity of the enzyme was divided by the weight of the bullfrogs.

RESULTS AND DISCUSSION

Figure 1 illustrates the average values of lipase activity of bullfrog chymes fed commercial feed for 87 days. The activity of this enzyme was not verified in the first two days of the experiment. The hydrolysis of the lipids in the diet was evidenced starting the third day, with the average frog weight of 4.25 g (Figure 2) and lipase activity of 3.46 UI. Lipase activity remained constant until the sixteenth day, with an average of the first fourteen-day period of 6.64 UI. Beginning on day eighteen, when animals reached average weight of 12.0 g, the lipase activity rose to 20.94 UI, which is an increase of 317% in relation to the previous period. It seems that bullfrogs need time, in the present case two weeks, to optimize lipase activity. This delayed appearance of lipase activity was also found for Nile tilapia, for which the activity was first detected in the brush border of the enterocytes, three days after hatch (Tengjaroenkul et al., 2002).

For bullfrogs within the thermal comfort for the species (27±1.0ºC), the digestion efficiency of the frogs in relation to feeds with ingredients rich in lipids begins to increase eighteen days after having concluded the metamorphosis process. Furné et al. (2005), demonstrated the fat digestion capacity of the Adriatic sturgeon (*Acipenser naccarii*) and the rainbow trout (*Oncorhyncus mykiss*), carnivorous fishes.

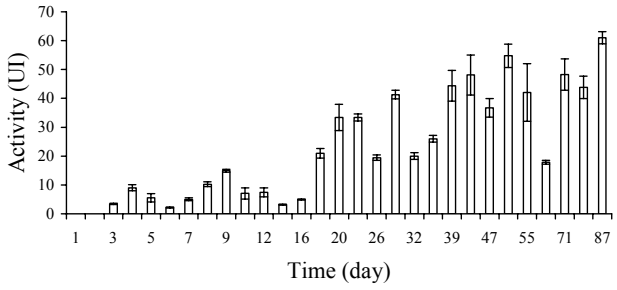

Figure 1 - Profile of the lipase activity of the chyme of frogs fed with commercial feed.

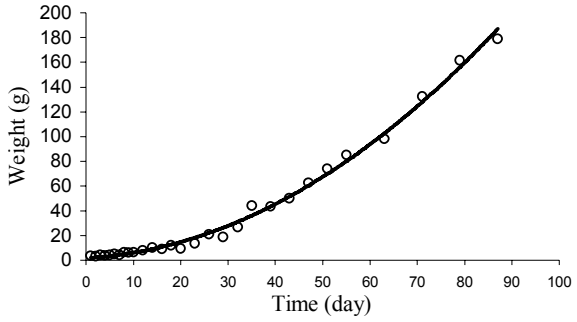

Figure 2 - Value of the bullfrog weight, for 87 days of experiment, fed commercial feed.

The presence of lipids in the feed probably stimulated the secretion and performance of the lipase enzyme. This fact was already described by Mukhopadhyay & Rout (1996) evaluating the effect of the supplementation of lipids (sunflower oil and cod oil) in iso-proteic and iso-energetic diets to carps (*Catla catla*). Lundstedt et al. (2004) also verified the effectiveness of diet formulations on lipase activity in the intestine of *Pseudoplatystoma corruscans* juveniles, a carnivorous animal similar to the bullfrog. The lipid source can also affect animal's performance as Morais et al. (2006) demonstrated for *Solea senegalensis* larvae.

The last sixteen samples were collected starting from day eighteen and are characterized by an increase in the activity of lipase in the chyme of the bullfrog. In only four samples values were detected below this tendency. The daily growth rate was 0.58 g day^{-1} when the frogs with an average weight of 179 g reached a lipase activity of 61.02 UI. In other words, as the animals gained weight, the capacity of the lipase to act in the hydrolysis of lipids also increased. The average activity of this period was 556% greater than that of the first stage, when the frogs reached medium weight of 9.36 g. The amount of lipids in the diet (9.72%) can also have stimulated the lipase activity. This was also observed by Hoehne-Reitan et al. (2001), when working with increasing levels of lipid inclusion during the feeding of *Scophthalmus maximus.*

The average values of lipase specific activity in the chyme of the frogs are illustrated in Figure 3. Here the activity of the enzyme is shown to be linked with the protein concentration in the chyme. It should also be noted that lipase specific activity has four different stages. The first three represented by periods of

stability and the last characterized by an increasing tendency. In the first stage, between day three and eighteen, the lowest average (217 UI mg^{-1}) was obtained. The second stable stage, with an average value of 1,167 UI mg^{-1} and fifteen days of duration, was marked by a large increase of the lipase specific activity in relation to the first stage. During the last stable stage, between the 39th and 55th day of the experiment, when the frogs reached an average weight of 85 g, the specific activity increased 66.56% in relation to the previous stage, presenting an average of 1,943 UI mg^{-1}. Soon after day 55, the specific activity increased to a rate of 50 UI mg^{-1} day^{-1}. On the last collection the highest lipase specific activity of 3,102 UI mg^{-1} was obtained.

The changes in activity and specific activity of lipase in bullfrogs were also detected by Seixas Filho et al. (2000). They analyzed surubim (*Pseudoplatystoma coruscans*), with weights varying from 226 to 658 g.

The lipase activity as a function of bullfrog weight fed commercial feed is available in Figure 4. Changes of the enzyme activity can be observed as a function of the weight of the frogs in the first 18 days of experiment. This coincides with the period of lowest lipase activity. This is probably due to the low activity of the enzyme in this initial phase when compared with the subsequent stages of activity in the chyme.

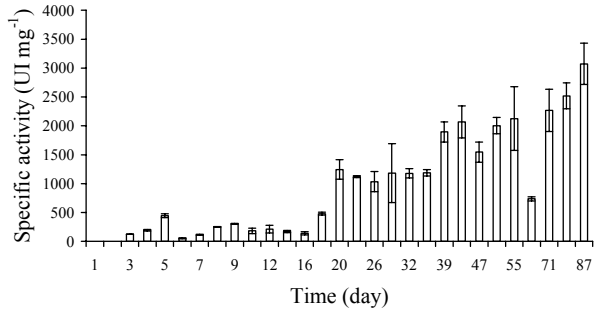

Figure 3 - Profile of the lipase specific activity of the chyme of frogs fed commercial feed.

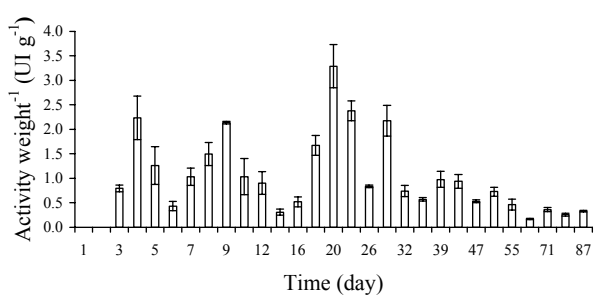

Figure 4 - Profile of the lipase activity as a function of bullfrog weight fed commercial feed.

Starting from the 20th day, when an average of 3.22 UI g^{-1} was obtained, the successive values of this parameter presented continuous reduction as the animals won weight. This tendency continued throughout the experiment when frogs with medium weight of 179 g presented 0.33 UI g^{-1}. I In other words, the increase of lipase activity was compensated by the growth of the frogs at a greater rate. This restricted capacity to digest lipids in the initial life phase of frogs was also verified by Braga et al. (1998), when measuring the nutritional value of soybean oil for frogs of 25 g. With the objective of increasing the quantity of energy of diets for juvenile bullfrogs, the results here obtained suggest that ingredients rich in fat (up to 5% inclusion) can be used. In feeds for adult animals, the proportion of these ingredients rich in fat can be increased beyond 5% to best fit their increasing capacity to digest lipids.

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