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Influence of supplying bullfrog tadpoles with feed containing 28% crude protein on performance and enzymatic activities[1]

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ABSTRACT - The objective of this study was evaluate the influence of feeding bullfrog tadpoles on commercial feed containing 28% crude protein (CP), on their digestive enzyme performance and activities. The experiment lasted 60 days, at the density of one tadpole/L in boxes containing 30 L water. A hundred and twenty tadpoles at the 25 Gosner stage averaging weight and length was 0.046 g and 6.22 mm, respectively, were used. Survival rate, length, final weight, weight gain, feed consumption, apparent food conversion, specific growth rate and activities of chime, amylase, lipase and trypisin were the parameters evaluated, biweekly, in five biometries. Quadratic effect was observed for the length and the weight. There was larger growth of the tadpoles from the 15th to the 45th day (19.82 mm). On the 15th day, the tadpoles presented the largest specific growth rate (16.93%/day), and the largest weight gain (5.460 g), feed intake (14.099 g) and the best apparent food conversion (2.46) was from the 45th to the 60th day. The specific activity of amylase was 205 times greater at 60 days when compared to the beginning of the experiment. The results demonstrated that, for the three enzymes studied, the action capacity over the tadpole chime increased significantly after the 30th experimental day. Moreover, they suggested a greater capacity of tadpoles to digest carbohydrates in detriment to proteins, and this fact was accentuated in the initial phase of the exogenous feeding of this amphibian. The commercial feed with 28% CP provide good performance in the bullfrog tadpoles, indicating the juvenile formation within commercial bullfrog farming standards.

Key Words: digestive enzyme, digestive physiology, frog farming, nutrition, performance

Influência do fornecimento de uma ração comercial com 28% de proteína bruta sobre o desempenho e a atividade de enzimas digestivas de girinos de rã-touro

RESUMO - Objetivou-se com este trabalho avaliar a influência da alimentação de girinos de rã-touro com ração comercial contendo 28% de proteína bruta (PB) no desempenho e nas atividades de enzimas digestivas desses animais. Utilizaram-se 120 girinos no estágio 25 de Gosner com 0,046 g e 6,22 mm, respectivamente, mantidos em quatro caixas contendo 30 L de água, na densidade de 1 girino/L. O experimento teve duração de 60 dias e quinzenalmente foram avaliadas as seguintes características de desempenho: taxa de sobrevivência, comprimento, peso final, ganho de peso, consumo de ração, conversão alimentar aparente, taxa de crescimento específico e as atividades no quimo das enzimas amilase, lipase e tripsina. A alimentação teve efeito quadrático sobre o comprimento e o peso final. O crescimento dos girinos foi maior do 15º ao 45º dia (19,82 mm). No 15º dia, os girinos apresentaram a maior taxa de crescimento específico (16,93%/dia), de modo que o maior ganho de peso (5,460 g), o consumo de ração (14,099 g) e a melhor conversão alimentar aparente (2,46) ocorreram do 45º ao 60º dia. A atividade específica da amilase foi 205 vezes maior aos 60 dias em comparação ao início do experimento. Os resultados indicam que, para as três enzimas em estudo, a capacidade de ação sobre o quimo dos girinos aumenta significativamente após o 3º dia experimental e que a capacidade dos girinos em digerir alimentos à base de carboidratos é superior à de digerir proteínas, fato que se acentua na fase inicial da alimentação exógena. A ração comercial com 28% de PB proporciona bom desempenho aos girinos de rã-touro, possibilitando a formação de imagos dentro dos padrões dos ranários comerciais, um indicativo provisório para alteração de manejo visando à redução de custos operacionais.

Palavras-chave: enzimas digestivas, fisiologia digestiva, girinagem, nutrição animal, ranicultura

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Introdução

In the tadpole phase, alterations in the production continuity are verified, by illnesses and mortalities in mass, caused by animal science technical procedures executed in empirical form, mainly inadequate food administration, because little is known about the nutritional requirements of the frogs (Seixas Filho et al., 1998a, b).

The relations between the feeding regimen and the characteristics of the digestive tract have been studied in other zoological groups, in which the technology has already reached a control stage in the formulation and manipulation of balanced feeds, indispensable to obtain satisfactory productive results (Seixas Filho et al., 2000a, b). However, the tadpole nutrition is far from establishing requirement standards that can be used by nutritionists.

To define the formulation of feeds compatible with the nutritional demands of bullfrog tadpole in the different phases of development, it is necessary to know the physiology of the this animal digestion. Efficient food use is directly related to the digestive process, in which enzymes are fundamental.

Generally, the bullfrog presents better performance when fed diets with high crude protein values, either in the tadpole phase (Carmona-Osalde et al., 1996; Albinati et al., 2000, 2001; Hayashi et al., 2004), the rearing (Braga & Lima, 2001) or finishing stage (Barbalho, 1991). On the other hand, protein levels between 26.6 and 33.6% are adequate for tadpole development (Barbosa et al., 2005), while 22.5% PB, in addition to not being adjusted, causes high mortality.

Bullfrog tadpoles fed commercial feeds with different crude protein levels (28, 32, 36, 40, 45 and 55% PB) do not present significant difference in the performance until the 25th day after the beginning of exogenous feeding, because there is not total functioning of the liver and pancreas (Seixas Filho et al., 2008a, b).

Information regarding the study of the enzymatic activity in amphibians (Etkin, 1968; Leone et al., 1976; Braga et al., 2004, 2006; Oliveira-Bahia, 2007) is scarce in the literature and lacks standardization of methodologies to supply satisfactory information to the nutritionists. Barbosa et al. (2005) mentioned that, in the literature, studies on the nutritional levels and their relations with growth are scarce. Some studies approach the effect of certain variables on tadpole growth, such as lightperiod (Bambozzi et al., 2004), the stocking density (Hayashi et al., 2004) and protein levels (Carmona-Osalde et al., 1996). A study seeking an excellent protein/energy ratio for bullfrog tadpoles was carried out by Seixas Filho et al. (1998b). The expense with frog feeding represents 57% of the total cost of the rearing (Lima & Agostinho, 1992).

Thus, this study was carried out with the objective of evaluating the influence of the feeding bullfrog tadpoles with commercial feed containing 28% crude protein in the performance and the enzymatic activities, basic evaluation so that nutritionists can define an feed standard and to adjust the different artificial feeding systems.

Material and Methods

The experiment was carried out in the Laboratory of Biological Research of the Grupo AQÜISUAM of the UNISUAM, Rio de Janeiro, and in the Laboratory of Enzymology of the Instituto de Biotecnologia Aplicada à Agropecuária – BIOAGRO of the Universidade Federal deViçosa, in Minas Gerais state.

During the 60 day experimental period, 120 bullfrog tadpoles (Lithobates catesbeianus Shaw, 1802) were used, 15 days old, at Gosner 25 stage (1960), proceeding from the same spawning of fishes.

The tadpoles were distributed in four white polyethylene boxes (62.5 × 40 × 16 cm) placed side by side on a bench, with 40 liter capacity, which received 30 L water, containing 30 animals with initial average weight and length of, respectively, 0.046 ± 0.011 g and 6.22 ± 0.68 mm, resulting in a density of one tadpole per liter, as described by Arruda Soares et al. (1985).

The water in the boxes renewed at 200% of the volume every 24 hours, with individual input, and kept at a constant temperature of 25.0 ± 1.0 °C. The water level inside the boxes was maintained by an "adapted knee" type device, attached to the side of the boxes, according to Seixas Filho et al. (1997), where the water flowed off through narrow PVC pipes placed laterally to the group of benches and connected to the sewer.

Each experimental unit received constant aeration, by puffer and 3/16'plastic hose, provided with porous rock at its extremity and regulated by a register of same caliber.

The commercial feed used in the feeding of the tadpoles (Table 1) was managed at the particle size of 0.5 mm, at the ratio of 10% of the body weight of the tadpoles per day and per box, and supplied once a day, at midday, as recommended by Seixas Filho et al. (1998a, b). The tadpole feed was moistened and placed in a corn form; after that, it was placed in feeding troughs placed on the bottom of the boxes, which consisted of 50 mm diameter PVC pipe, cut lengthwise and the size of the width of the box, kept fixed to its sides, without movement.

Table 1 - Level of guarantee and results of the laboratorial analysis of the commercial feed used in the feeding of bullfrog tadpoles (*Lithobates catesbeianus*)

Level of guarantee (%)	Value
Maximum humidity	13.0
Crude protein (CP)	28.0
Ether extract (minimum)	4.0
Fibrous substance (maximum)	10.0
Ash (maximum)	14.0
Calcium (maximum)	3.0
Phosphorus (minimum)	0.6
Examined composition	
Humidity	9.54
Dry matter	90.46
Gross energy, kcal/kg	4,098
Crude protein (CP)	30.88
Ether extract (minimum)	3.50
Mineral matter	12.65
Non-nitrogenous extract	60.57

Basic composition: soybean meal, fish meal, wheat bran, corn gluten meal 60%, meat and bone meal, corn, blood meal, fish oil, calcium carbonate, dicalcium phosphate, add salt and vitamin[1] mineral[2], anti-oxidant[3].
[1] Composition per kg: vit. A – 12,000 UI; vit. D_3 – 4,000 UI; vit. E – 150 UI; vit. K – 10 UI; ácido fólico – 10 mg; biotin – 0.8 mg; choline – 500 mg; niacin - 150 mg; calcium pantothenate – 50 mg; thiamine – 30 mg; riboflavin – 30 mg; pyridoxine B6 – 30 mg; vit. B12 – 35µg; vit.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.

The boxes were cleaned daily, in the morning (at 8 a.m.), by bottom siphonage, removing the feed excrements and remaining portions. Daily, in the morning and afternoon, the air and water temperatures were taken with a mercury column thermometer (0 to 60 °C). The ammonia and pH were controlled daily, by colorimetric means, using a commercial kit.

The biometrias were carried through biweekly for evaluation of final weight, length and survival of all the population of tadpoles. The animals were placed on a moistened cloth towel, to remove excess water, without abrupt dehydration, and measured from the mouth to the insertion of the tail, with a digital caliper, with a hundredth of a millimeter precision. After that, each animal was transferred to a plastic recipient with approximately 5 mL of water and weighed on an analytical scale, with of 0.001 g precision, previously weighted.

To evaluate feed intake, the surplus feed distributed to the animals was removed from the feeding troughs every 24 hours, by siphonage, with a 3/16' diameter hose and filtering, by means of a filter with a 0.5 mm mesh, not contaminated with excrements. The leftovers were dried in a greenhouse ventilated at 55 °C, for 24 hours and, after removal from the greenhouse, they were left for one hour to balance with the environment and weighed on a digital scale with 0.001 g precision. The intake was calculated by the difference between the offered amount and leftover feed.

The apparent food conversion was obtained by the ratio between the feed intake and weight gain. The weight gain was calculated by the difference between the weights of two consecutive biometrias. The expression following was used to determine the specific growth rate (SGR):

$$TCE = \frac{\text{log natural of the final weight (g)} - \text{log natural of the initial weight (g)} \times 100}{\text{Experimental period (days)}}$$

For the analyses of the digestive enzymes in the chime of the tadpoles, the digestive tract was isolated of the animals and the segments analyzed in the Laboratory of Biological Research of the Grupo AQÜISUAM of the UNISUAM (Figure 1).

Four units of each treatment were collected, biweekly, in each biometry. For collection, the animal metabolism was reduced by immersion in an ice bath at -4º C, and anaesthetized with 1% menthol added to the water. After that, they were contained physiologicaly and submitted to autopsy for withdrawal of the intestine.

The autopsy of the bullfrog tadpoles was carried out on an ice bed (Figure 1A), which consisted of a completely frozen container; a sheet of aluminum paper was placed under the ice rock that the water did not come in contact with the intestine wall and modify the chemical structure of the chime, called an "autopsy bed".

The beginning of the autopsy consisted of placing the animal on the autopsy table, with the support of a clamp placed inside the animal mouth and another one at the beginning of the insertion of the tail (Figure 1 B) so that it had resistance to the scalpel incision.

The animals suffered longitudinal ventral incision (Figure 1C) and the digestive tube was isolated, double ties in the cranial portion of the esophagus and in the tail portion of the rectum, before the anus, so that the chime did not leak. After the complete incision, the intestine could be visualized, with its handles forming a circular topography, in centripetal and centrifugal directions (Figure 1D). Using an oblique spatula, the intestine was removed from the peritoneal cavity, isolated from the other organs. After the isolation, the intestine was parted in the cranial portion, right after manicoto, that is, in the pyloric region of the digestive tract (Figure 1E). The tail portion was sectioned before the anus. They were placed in polyethylene bottles and stored in a freezer at -8 ºC until the enzymatic activity analyses (Figure 1F).

The enzymatic activities were analyzed in the Laboratório de Enzimologia e Bioquímica de Peptídeos of the Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO) of the UFV.

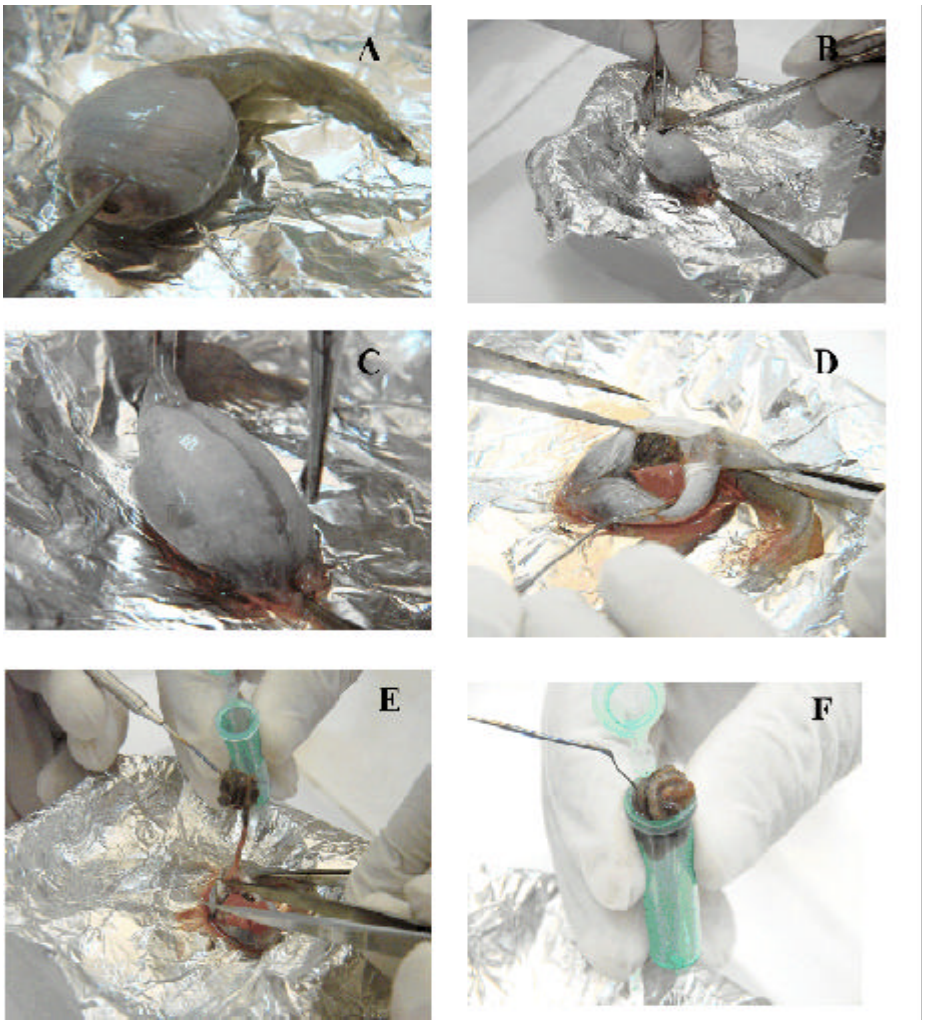

Figure 1 - Details of the beginning of the autopsy of the bullfrog tadpole (A); placing a clamp in the mouth and another one in the tail (B); initial incision in the ventral region (C); aspect of the topography of the intestinal handles (D); withdrawal of the intestine from the peritoneal cavity (E); preservation of the intestine with chime (F).

For the amylase, lipase and trypsin analyses , 0.5 g of each frozen sample and 1.5 mL drain plug were used (Tris-0.1 HCl M), to defrost and macerate. The material was then centrifuged at 15,000 rpm for 15 minutes at 4 ºC and the supernatant was used to determine the activities in duplicate.

The amylase activity was determined on the basis of starch hydrolysis by amylase, with release of glucose and dextrin molecules. Because of the iodine addition, the non-hydrolyzed starch acquires blue coloration. Amylase activity is inversely proportional to the intensity of the blue color and calculated by comparison to a substratum control. The activity was determined in an optical spectrophotometer, at 660 nm wave length, using the Bioclin colorimetric amylase kit, according to Caraway (1959). The specific amylase activity was obtained by dividing the value of the amylase activity by the value of the starch concentration of the respective sample.

The lipase activities were determined using the Bioclin kit with modified methodology by Cherry & Crandall (1932), that consists of verifying the performance of chime lipase on an glycerol ester, which liberates a chromogen quantitatively determined at 410 nm. The intensity formed is proportional to the lipase activity and the values are expressed in International Unidades (UI).

The trypsin activities were determined by the method described by Erlanger et al. (1961) using N-Benzoil-D, p-nitroanilida L-arginine (D, L-BApNA) as substratum. Initially, a solution with 0.5mL was made from this substratum with 0.5mL Tris-HCl Drain plug 0,1M, pH 8,0, in test tubes. Ten µL were added of the supernatant and, immediately, the initial speeds were determined by the formation of the product p-nitroalinine, by determining absorbance at 410 nm, in function of time. For the calculations, the molar extinguishing coefficient of 8800 M-1 cm-1 was used for the product.

The protein concentration, for all the samples of different enzymes, was measured in an optical spectrophotometer at 280nm absorbance. The presence of nucleic acid caused interferences in the optic readings, problem that was solved making a reading at 260 nm, because the nucleic acids absorb the light more strongly at this wave length. The ratio of these two values was determined and used to select a correction factor. The absorbance of 280 nm was multiplied by this factor and, with this, the protein concentration, in mg mL^{-1}, was obtained (Warburg & Christin, 1941).

The data of weight, length, gain of weight, feed intake, food conversion, and the activities of amylase, lipase and trypsin enzymes, and their specific activities were submitted to regression analysis. Linear models of each parameter in function of the experimental period were tested and the choice of the best equation was given by the greatest coefficient of determination (R^2), for the significance of the coefficients of regression by t test at 5 and 1% probability and for its biological adequacy.

Results and Discussion

The air temperature presented minimum and maximum average of 26 ± 1 °C and 29 ± 1 °C, respectively. The average of the water temperature observed varied between 25 ± 1 °C. The pH remained in the range of 7.6 ± 0,6 and the total ammonia varied between 0 to 0.25 ppm, which, when compared by the scale of the kit for aquariofilia, remained within the desired. The temperature and pH values remained within the acceptable limits for the tadpoles and were similar to the conditions observed by Braga & Lima (2001), confirming reports by Tavares (1994) that good quality water in tanks and fisheries is the key for success of the rational aquiculture production.

The survival rate was of 85% at the end of the experimental period (Table 2; Figures 2 and 3). These results were similar to ones obtained by Seixas Filho et al. (1998 b), who observed 82% survival for bullfrog tadpoles fed feed containing 35% CP and 4200 kcal/kg crude energy and inferior to the 97% of Albinati et al. (2001) in tadpoles fed feed containing 35.61% CP and 3,954 kcal/kg digestible energy. On the other hand, they were superior to the results reported by Stéfani et al. (2001) and Barbosa et al. (2005), who observed survival rates of 60% and 50% when feeding tadpoles feed with 35.03% CP and 4173,59 kcal/kg EB and isocaloric feeds of 22.5 to 33.6% CP and 4,100 kcal/kg EB, respectively.

The feeding had a quadratic effect ($P<0.01$) on the final weight and the length throughout the experimental period (Table 2). The growth of the tadpoles was greatest between the 15th and 45th days (19.82 mm) and smallest (4.33 mm) at the end of the experiment (from 45th for 60th day).

In first the 15 days and considering the physiological adaptation to the supplied food (feed), the animals presented the smallest weight gain (0.536 g) in relation to the other periods. However, the specific growth rate was greater (16.93% /day), which can be associated to the more accented metabolism of the species in this same phase.

The reduction in growth from the 45th to the 60th day of the experiment can be related to the pre-metamorphic phase, when the animals exteriorize their posterior members. However, during this phase, the biggest weight gain (5,460 g) is related to the development of the members and the improvement in the intake (14.099 g) and in the apparent food conversion (2.64). In the interval of 15 to 30 days, the tadpole performance improved, as a result of the development of the digestive system, increasing the use of the nutrients of the diet. On the other hand, Albinati et al. (2001)

Table 2 - Performance of bullfrog tadpoles fed commercial feed containing 28% crude protein (CP) for 60 days

Parameter	Period (days)				
	0	15	30	45	60
Tax of survival (%)	100	95.56	94.17	91.67	85.00
Length (mm)[2]	6.22	13.09	24.55	32.91	37.24
Final weight (g)[2]	0.046	0.583	3.140	6.019	11.479
Weight gain (g)[1]	-	0.536	2.557	2.879	5.460
Feed intake (g)[2]	-	3.701	8.456	15.694	14.099
Food conversion	-	7.09	3.44	5.56	2.64
Specific growth rate (%/day)	-	16.93	14.08	10.83	9.20
Amylase (U.A)[2]	5.29	359.57	638.18	686.39	504.20
Specific Amylase activity (U.I./mg)[2]	0.51	61.89	60.77	74.99	104.54
Lipase (U.I)[3]	105.24	185.21	134.73	154.04	160.46
Specific lipase activity (U.I./mg)[2]	10.22	28.15	13.84	15.70	33.75
Triypsin ($nM.s^{-1}$)[2]	1.92	1.85	9.88	25.02	47.26
Specific tripsin activity ($nM.s^{-1}/mg$)[2]	0.19	0.66	0.80	3.27	8.09

[1] Linear effect. [2] Quadratic effect. [3] Not-significant effect.

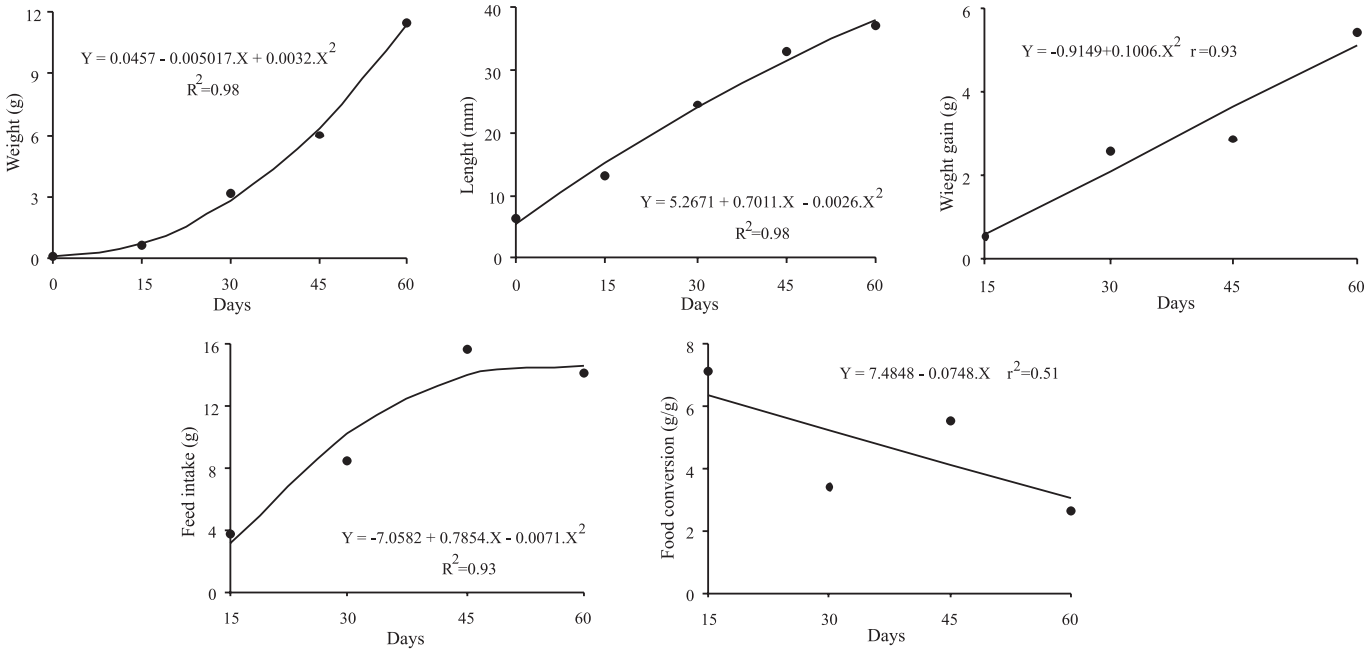

Figure 2 - Weight, length, weight gain, feed intake and apparent food conversion of the bullfrog tadpoles fed with commercial ration I contend 28% of crude protein.

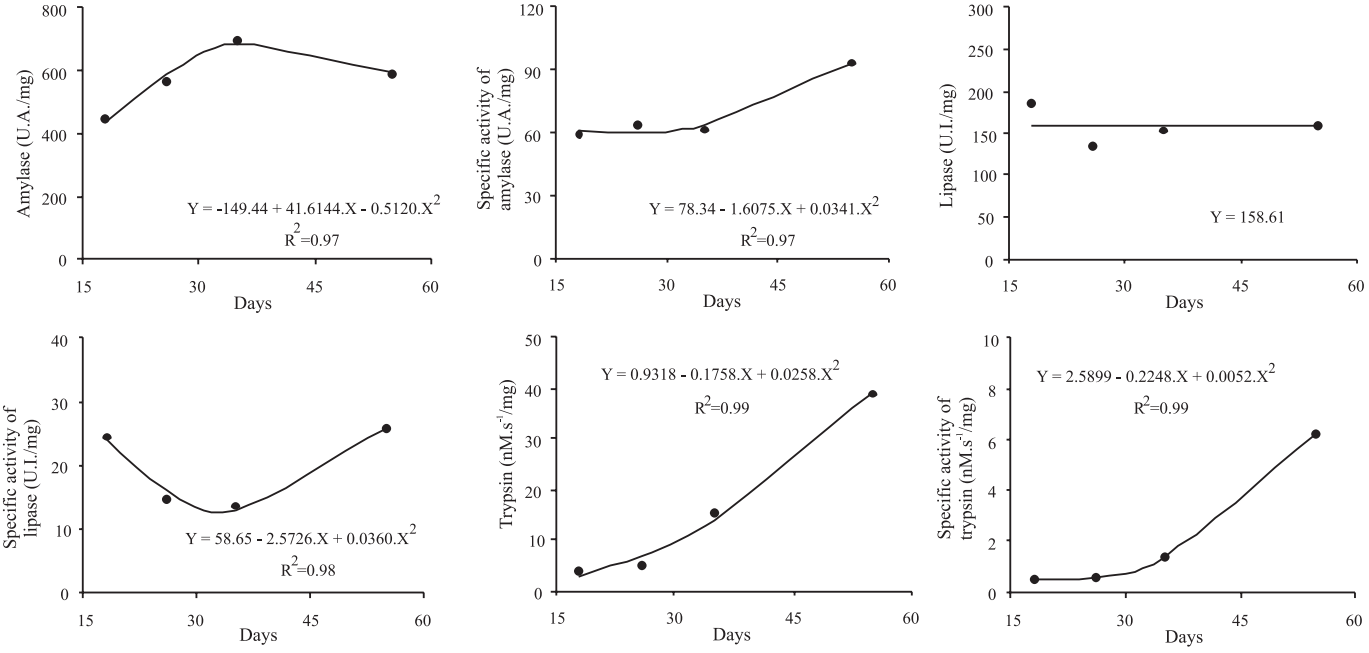

Figure 3 - Enzymatic activity and specific activity of amylase, lipase and trypsin in chime of bullfrog tadpoles fed with commercial feed containing 28% crude protein.

obtained better results in weight gain and food conversion when using feed with 38.6% CP and 3,954 kcal/kg digestible energy, although working with a population density six times smaller the one used one in the present study.

Stringuetta et al. (2007) studied the use of tilapia filleting residues in feed for tadpoles with 33.5% crude protein, in a density of 2.5 tadpoles per liter, obtained smaller weight gain, though with better food conversion. However, Barbosa et al. (2005) observed poorer performance

of the tadpoles fed feeds containing different protein levels (22.5, 26.6 and 33.6% of CP) in meso-scale experimentation.

The lack of standardized methodology for consistent data and the scarcity of information in the literature on the tadpole nutrition harm the comparison of results between the published works.

The activity of amylase, lipase and trypsin was verified since the first collection day of the chime of tadpoles fed commercial feed containing 28% crude protein (Table 2 and Figure 3). These results suggest that the tadpoles can use the carbohydrates, the fat and the proteins from artificial diets.

The development phases had a quadratic effect ($P<0.05$) on the specific amylase activity (Table 2). At the end of first 15 days of the experiment, when the tadpoles presented were at stage 26 (Gosner, 1960), pre-metamorphic phase, the specific activity already reached 61.89 U.A./mg CP, remaining at 60.77 U.A./mg CP on the 30th day, when the animals were at stages 28 to 31, the end of the pre-metamorphic phase and beginning of the post-metamorphic phase (Gosner, 1960), growing in the 45th day (74.99 U.A./mg CP) with the tadpoles at stages 35 and 36, half of the post-metamorphic phase (Gosner, 1960), and, even more, on the 60th day (104.54 U.A./mg CP), in the end of the post-metamorphic phase (Gosner, 1960), when the tadpoles were at stages 37 to 39. With this, by the end of the experiment, there was an approximate 205-fold increase in the starch hydrolysis capacity in comparison to the initial capacity (0.51 U.A./mg CP).

The results of this study are in accordance with those reported by Leone et al. (1976) who analyzed the profile of the enzymatic activity of amylase and lipase of the tadpoles of *Xenopus laevis* and observed that the enzymes had promoted a peak of activity between stages 29 and 38, greater level of ultrastructural organization of the acinar cells, corresponding in the present study to the period from the 30th to the 60th day. They match the histological studies of the liver and the pancreas carried out by Seixas Filho et al. (2008a).

The increase in the amylase hydrolytic capacity observed on the 60th day is in accordance with reports by Etkin (1968) who stated that at the stages of 36 to 40 (pro-metamorphosis phase), the biggest body growth of the tadpoles occurs, leading to greater nutrient intake and consequently, a bigger contribution of enzymes if it becomes necessary. Similar results were obtained by Oliveira-Bahia (2007) who reported high increase in amylase activity from stages 36 to 40 in the pancreas of bullfrog tadpoles, that corresponds, in the present study, to the last experimental fortnight.

The feed had quadratic effect ($P<0.05$) in the specific lipase activity, which was bigger in the tadpoles in the first 15 days (Table 2 and Figure 3) and can be explained by the fact that the tadpoles have high metabolism in this phase, which requires greater energy levels to support its development, and consequently there is a bigger enzyme contribution.

Over 30 days, with the reduction in the growth rate, the level of energy required for the development process also diminished, proving that the lipase activity was regulated physiologically by the tadpoles.

From the 45th to the 60th day of the experiment, with the arrival of the pre-metamorphic phase, the specific lipase activity increased, probably in virtue of the better functioning of the pancreas and the requirement of more energy for the development of the posterior members at this age, matching the reports by Etkin (1968), Seixas Filho et al. (2008a) and Oliveira-Bahia (2007).

In the evaluation of the specific trypsin activity, at the beginning of the experiment, there was low capacity for substratum hydrolysis (0.19 $nM.s^{-1}$/mg) in comparison to the that of other enzymes (Table 2). At 15 days of experimentation, there was little increase in the hydrolysis capacity, of the order of 3.5 times (0.66 $nM.s^{-1}$/mg) compared to the initial.

At the 30th day occurred a discrete 4.2-fold increase (0.80 $nM.s^{-1}$/mg) in relation to the initial capacity and of only 0.23 fold in relation to the previous fortnight. However, from the 45th day, there was increase (3,27 $nM.s^{-1}$/mg) in the hydrolytic capacity of this enzyme of 17 times the initial capacity and of 4,0 times in relation to the last fortnight (Table 2).

By the end of the experiment the specific trypsin activity was 8.09 $nM.s^{-1}$/mg, corresponding to 42.5 times the initial hydrolysis capacity and 2.47 times bigger than the previous analyzed period, matching the reports by Oliveira-Bahia (2007).

These results prove that, the three enzymes under study promote significant increase in the capacity of action on chime of the tadpoles after the 30th day of experimentation, which matches the reports by Seixas Filho et al. (2008 a, b) corroborating those by Leone et al. (1976) who stated that, in *Xenops laevis* tadpoles, only from stage 29 (Gosner, 1960), is there complete maturity of the structures responsible for enzyme synthesis and secretion, corresponding, in the present study, to the period from thef 15th to the 30th day after the beginning of exogenous feeding.

These results further suggest that tadpoles have a greater capacity for digesting foods with carbohydrate

base in detriment to protein, and this fact was accented in the initial phase of the exogenous feeding of this anurous.

The optimum performance of the animals in the last fortnight of the experiment was directly is related to the highest hydrolytic capacity of the enzymes, which reflected in the increased weight gain with the best apparent food conversion (2.64), causing a final weight of 11.479 g. This final weight is considered good for the animals that are still in the pre-metamorphic phase, indicating the formation of juvenile bullfrogs within the standards of commercial frog breeding, with lower operational cost in the feeding, as indicated by Lima & Agostinho (1992).

On the other hand, the knowledge of the morphophysiological alterations is fundamental for the comprehension of the mechanisms inherent to the digestion of nutrients in each phase of tadpole development and for the supply of pertinent information on the nutrition and handling of these animals in captivity.

Conclusions

The three enzymes studied promoted significant increase in the action capacity on the chime of the tadpoles after the 30th experimental day. The capacity of the tadpoles to digest foods to with carbohydrate base is superior to their capacity to digest proteins, a fact accented in the initial phase of exogenous feeding. The commercial feed with 28% crude protein provide good performance in the bullfrog tadpoles, which indicate the formation of juvenile bullfrogs within the standards of commercial frog breeding, a provisory indicative for handling alteration aiming the reduction of operational costs.

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