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FEEDING PROGRAMS FOR BULLFROG (*Lithabates catesbeianus*-*Rana catesbeiana*, Shaw 1802)

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ABSTRACT

We used 525 bullfrog tadpoles, distributed into 15 boxes with one tadpole per liter. The water was renewed daily (200%). The feeding regimes (FR) were constituted of five arrangements using seven commercial rations with levels of 22, 28, 32, 36, 40, 45 and 55% of crude protein (CP), supplied every 15 days. We used a random blocks design, and subdivided plots with three replications. The subplots were constituted of five biometries: at the beginning of the experiment, at 15, 30, 45, and 60 days. The tadpoles submitted to feeding regime FR1, with 22, 32, 36, and 40% of CP, presented the highest consumption and the same performance as the others. Tadpoles that received FR5, with 40, 45, 45 and 50% of CP, presented greater weight; however, they showed smaller weight gain, greater consumption, conversion and mortality, indicating that this regime is expensive and not adequate for management. Feeding regimes FR2, with 28, 32, 36 and 40% of CP, and FR3, with 32, 36, 40 and 45% of CP, were more adjusted to this kind of management. Animals in all treatments showed higher performance than animals fed traditionally.

KEYWORDS: bullfrog nutrition; nutritional requirement; protein.

REGIME ALIMENTAR DE GIRINOS DE RÃ TOURO

RESUMO

Foram utilizados 525 girinos de rã-touro durante 60 dias, distribuídos em 15 caixas, com um girino por litro. A água foi renovada 200%, a cada 24 horas. Os regimes alimentares foram elaborados por meio de cinco arranjos elaborados com sete rações comerciais com níveis de 22, 28, 32, 36, 40, 45 e 55% de proteína bruta (PB), ministradas a cada 15 dias. Foi utilizado o delineamento em blocos ao acaso, em parcela subdividida, com três repetições. As subparcelas foram constituídas por cinco biometrias: na instalação do experimento, aos 15, 30, 45 e 60 dias. Os girinos submetidos aos regimes alimentares RA1, com 22, 32, 36, e 40% de PB, apresentaram o maior consumo e o mesmo desempenho dos demais. Já os que receberam o RA5, com 40, 45, 45 e 50% de PB, apresentaram maior peso, mas o menor ganho de peso, maiores consumo, conversão e mortalidade, indicando que esses regimes oneram e não foram adequados ao manejo. Os regimes alimentares com 28, 32, 36 e 40% de PB e com 32, 36, 40 e 45% de PB foram mais adequados a esse tipo de manejo. Os animais, em todos os tratamentos, alcançaram desempenho maior que os alimentados tradicionalmente.

PALAVRAS-CHAVE: exigência nutricional; nutrição de rã; proteína.

INTRODUCTION

The aquatic phase of tadpoles rearing is the basis for obtaining a good result from frog farming (ALBINATI et al. 2000), where high losses or poor performance of animals, generally showing signs of nutritional deficiencies, are recorded (SEIXAS FILHO, 2011a; BARBOSA et al., 2005), associated with water quality, as reported by FONTANELLO et al.1982). According to CASTAGNOLLI (1992) and TAVAREZ (1994), water quality includes all physical-chemical-biological characteristics that may affect its use, without interference in research results.

LIMA & AGOSTINHO (1992) reported that the cost of feeding frogs represents 57% of the total cost of breeding. SEIXAS FILHO et al. (2011a) stated that the main problem for a full breeding is related to the composition of properly nutritional food, regarding particularly the protein content, due to lack of knowledge of the nutritional requirements of these animals.

In Brazil, especially in Rio de Janeiro, the market for frog meat is still small but stable, and all frog breeders produce their own tadpole, using commercial feed for fish throughout the larval period, with single protein level, usually high, increasing operational costs and deteriorating water quality (SEBRAE-RJ, 2002).

SEIXAS FILHO et al. (2011b) evaluated the gastrointestinal tract of bullfrog's tadpoles and observed the presence of liver cells with more compact glandular arrangement only on the 25th day after the feeding period, suggesting functionality, which allows inferring that feeding management with high nitrogen level from the beginning of breeding can lead to toxicity in animals due to lack of physiological conditions for metabolism, causing mortality, reduced growth and population heterogeneity.

HIPOLITO et al. (2001) stated that the whole performance of the bullfrog is based on supply and any change in liver function can compromise the entire protein and nutritional use. The same authors examined the liver of bullfrogs fed commercial diets, and observed, in most cases, vacuolization and disruption of cell contour, rarefaction and hydropic cell degeneration, and histopathological features associated with protein deficiency, which may be due to poor quality or low protein utilization by the animal.

Therefore, this study aimed to evaluate a new methodology for feeding tadpoles at this stage of breeding, by testing different diets.

MATERIAL AND METHODS

We carried out the experiment at the Biology Research Laboratory of UNISUAM - Rio de Janeiro - RJ.

We used 525 bullfrog tadpoles *Lithabates catesbeianus,* new name of *Rana catesbeiana* (FROST et al., 2006), at 15 days, beginning of exogenous feeding, stadium 25, according to GOSNER (1960), during the experimental period of 60 days. We used commercial diets as feed for the tadpoles, with seven levels of crude protein (CP) – 22%, 28%, 32%, 36%, 40%, 45%, and 55% – (Table 1), with a particle size of 0.5 mm, administered at the rate of 10% of the live weight of the tadpoles, distributed once a day at 12:00 p.m., according to SEIXAS FILHO et al. (2011b). We distributed the animals into 15 plastic boxes with a capacity of 50 liters each, with 35 liters, placed side by side on a bench, containing a PVC trough, where the feed was concentrated, and at a density of one tadpole per liter of water, maintained throughout the experiment, removing a liter of water for every dead animal (ARRUDA SOARES et al., 1983).

The renewal of the water in the boxes, kept at a constant temperature of 25°C (± 1 °C), was approximately 200% of the volume every 24 hours, drained through PVC channel drains placed alongside the counter and connected to the sewer. The volume of water was maintained by a "knee"-type device adapted to boxes according to SEIXAS FILHO et *al.* 1997.

Each experimental module received constant aeration by means of blower and 3/16 plastic hoses, with porous stones at their ends and regulated by a valve of the same size.

Table 1 - Commercial Diets with 22%, 28%, 32%, 36%, 40%, 45% and 55% crude protein (CP) fed to bullfrog tadpoles (*Lithabates catesbeianu*)

Warranty levels	Ration (% PB)						
	22	28	32	36	40	45	55
Maximum humidity	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Crude protein (CP)	22.0	28.0	32.0	36.0	45.0	55.0	32.0
Ether extract (minimum)	4.0	4.0	7.0	8.0	8.0	10	7.0
Fibrous matter (maximum)	10.0	10.0	7.0	7.0	7.0	5.0	7.0
Ashes (maximum)	14.0	14.0	2.6	3.1	3.1	3.0	2.6
Calcium – Ca (máximo)	3.0	3.0	12.0	14.0	14.0	14.0	12.0
Phosphorus – P (mínimo)	1.0	0.6	2.0	2.5	2.5	2.0	2.0
Basic Composition							
Soybean meal, fish meal, wheat meal, corn gluten meal 60%, meat and bone meal, corn, blood meal, fish oil, calcium carbonate, dicalcium phosphate, salt, vit.[1] and mineral [2] supplement, antioxidant[3].							

[1]Composition per kg: VIT. A - 12,000 UI; VIT. D_3 - 4000 UI; VIT. E - 150 UI; VIT. K - 10 UI; Folic acid - 10mg; Biotin - 0.8 mg; Choline - 500 mg; Niacin - 150 mg; Calcium pantothenate - 50mg; Thiamin - 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 feeding management was designed to allow the gradual introduction, every 15 days, of increasing levels of nitrogen in animal metabolism through arrangements among the seven commercial diets (Table 2).

The feed was provided to tadpoles in troughs which consisted of a 50 mm PVC tube, as long as the width of the box, and cut along the length. The troughs were fixed on the sides of the experimental module, to remain still (Figure 1).

Table 2 - Crude protein levels (%) of the commercial feed offered to bullfrog tadpoles for 60 days under different feeding regimes

Period	Feeding regimes				
(days)	FR1	FR2	FR3	FR4	FR5
0 – 15	22	28	32	36	40
15 – 30	28	32	36	40	45
30 – 45	32	36	40	45	45
45 – 60	36	40	45	45	55

[1] FR1 - 22, 28, 32 and 36% CP; FR2 - 28, 32, 36 and 40% CP; FR3 - 32, 36, 40 and 45% CP; FR4 - 36, 40, 45 and 45% CP; FR5 - 40, 45, 45 and 55% CP offered every 15 days.

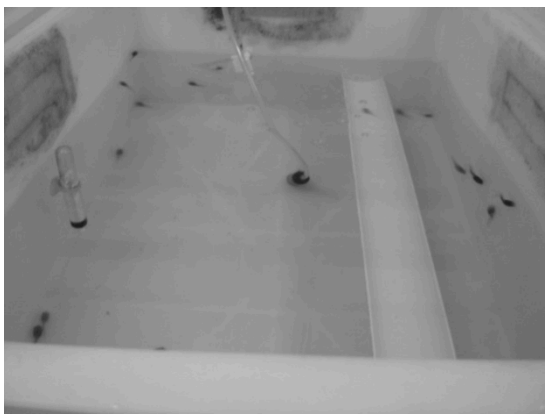

Figure 1 - Positioning of pvc trough in the experimental module for bullfrog tadpoles *(Lithabates catesbeianu)* feeding

We cleaned the boxes every day by siphoning the bottom, removing feces and food remains. We measured the temperatures of air and water daily, in the morning and in the evening, by a mercury thermometer with Celsius scale, 0 to 60 °C. We controlled ammonia and pH daily by means of titration and a digital device, respectively. We used a kit to control pool water every week.

We carried out biometry every two weeks, evaluating weight, length and survival. We placed the tadpoles on moistened cloth towel to remove water excess, without any abrupt dehydration, and measured them from the mouth to the insertion of the tail, with the aid of a digital caliper with a precision

of hundredths of a millimeter. Subsequently, we transferred each animal to a plastic container with a volume of approximately 5 mL of water and weighed them on an analytical balance, adjusted to an accuracy of 0.001 g.

For intake evaluation (I), we removed from the troughs the leftovers of the feed offered to the animals every 24 hours by siphoning with the aid of a hose with a diameter of 3/16', and filtering through a filter of 0.5 mm mesh without feces contamination. The leftovers were dried in a ventilated oven at 55°C for 24 hours. After removal from the oven, they were left for an hour to come to balance with the environment and were, then, weighed on a digital scale with a precision of 0.001 g. Intake was then obtained by the difference between the amount offered and the leftover.

The apparent feed conversion ratio (AFC) was calculated by dividing feed intake (I) and weight gain (WG). Weight gain was calculated as the difference between the weights of two consecutive biometry evaluations.

We used a randomized block design in a split-plot layout with three replications. The plots were the feeding regimes (FR) (Table 1) and the subplots were the five biometry evaluations: at the installation of the experiment (initial), and at 15, 30, 45 and 60 days after installation. Data were subjected to analysis of variance and F test. The diets were compared using the Newman-Keuls test at 5% probability.

RESULTS AND DISCUSSION

Air temperature presented minimum and maximum averages of 25 and 30 °C, respectively. Water temperature average ranged from 24 to 26 °C. The pH remained in the range of 7.0 to 8.9, and the total ammonia varied from 0 to 0.25, which was within the recommended range when compared to the scale of the aquarium kit. Temperature and pH values were within acceptable limits for the tadpoles, and were similar to the conditions of the studies of FONTANELLO et al. (1982) and SEIXAS FILHO et al. (1998 b), and in accordance with TAVARES (1994), who stressed that good quality water in tanks and ponds is the key to success of rational production of aquaculture.

Tadpoles subjected to FR5, with 40, 45, 45 and 50% CP, showed significant differences ($P < 0.05$) compared to other treatments, with the highest mean weight at 60 days (Table 3). However, they showed the lowest weight gain and the highest feed conversion (Table 4), besides higher mortality (34.3%), according to the results displayed in Table 5. This indicates that this regime was not suitable for this phase of growth of tadpoles, according to the findings by SEIXAS FILHO et al. (2008).

Table 3 - Weight and average length of bullfrog tadpoles fed commercial diets with different crude protein (CP) levels for 60 days

	Biometry (days)									
	0	15	30	45	60	0	15	30	45	60
	----------		Weight (g)	----------			----------	Length (mm)		
FR1	0.033 a	0.39 a	2.00 a	6.22 b	11.31 ab	5.4 a	11.3 a	20.3 b	31.4 ab	41.2 a
FR2	0.032 a	0.57 a	2.49 a	6.32 b	11.72 ab	5.5 a	12.7 a	20.9 ab	30.9 ab	40.7 a
FR3	0.032 a	0.55 a	2.23 a	5.65 b	10.76 b	5.3 a	12.8 a	22.4 ab	30.4 b	39.8 a
FR4	0.033 a	0.74 a	2.75 a	7.83 a	12.54 ab	5.5 a	13.8 a	22.9 ab	33.3 ab	41.3 a
FR5	0.033 a	0.73 a	3.81 a	8.91 a	13.17 a	5.6 a	14.2 a	24.3 a	34.9 a	42.1 a

In each biometry evaluation, means followed by at least one letter do not differ by Newman-Keuls test (P> 0.05).
[1]FR1 - 22, 28, 32 and 36% CP; FR2 - 28, 32, 36 and 40% CP; FR3 - 32, 36, 40 and 45% CP; FR4 - 36, 40, 45 and 45% CP; FR5 - 40, 45, 45 and 55% CP offered every 15 days.

The other feeding regimes showed no significant differences among each other (P> 0.05) for length, weight gain and feed conversion at 60 days, indicating that, for these parameters, any of them could be suitable for the management of these animals.

On the other hand, FR1, with 22, 28, 32 and 36% CP, showed higher feed conversion than the others, at the first 15 days of the experiment. Because of this, this feeding regime is not recommended because, despite the low protein level, it showed the need to use a larger amount of feed in order to achieve similar weight gain to the animals fed the other FR, presenting worse index of feed efficiency (IFE), which is the average weight gain per animal on the lot divided by the average feed intake per tadpole. This implies an increase in operating costs of rearing, besides a possible functional alteration of the liver, as reported by HIPOLITO et al.2001.

According to LIMA & AGOSTINHO (1992), the cost of feeding for frogs represents 57% of the total rearing costs. Thus, FR2 and FR3, with 28, 32, 36 and 40% CP, and 32, 36, 40 and 45% CP, respectively, seem best suited to this type of management, with lower operating costs, considering FR3 and FR4 (with 32, 36, 40 and 45% CP, and 36, 40, 45 and 45% CP, respectively) showed no significant difference (P> 0.05) between each other.

Table 4 - Weight gain and mean apparent feed conversion of bullfrog tadpoles fed commercial diets with different crude protein (CP) levels for 60 days

	Periods (days)							
	0 – 15	15 – 30	30 – 45	45 – 60	0 – 15	15 – 30	30 – 45	45 – 60
	Weight gain (g)				Apparent feed conversion			
FR1	0.359 a	1.566 b	4.216 ab	4.816 a	4.57 a	2.06 a	1.04 a	1.89 b
FR2	0.535 a	1.837 b	3.776 b	5.088 a	3.01 b	1.89 a	1.26 a	1.87 b
FR3	0.520 a	1.644 b	3.314 b	4.869 a	3.11 b	1.83 a	1.18 a	1.88 b
FR4	0.704 a	1.905 ab	5.073 a	4.451 a	2.40 b	1.87 a	0.92 a	2.13 b
FR5	0.692 a	2.901 a	5.024 a	3.361 b	2.80 b	1.54 a	1.19 a	3.72 a

In each biometrics evaluation, means followed by at least one letter do not differ by Newman-Keuls test (P> 0.05).
[1]FR1 - 22, 28, 32 and 36% CP; FR2 - 28, 32, 36 and 40% CP; FR3 - 32, 36, 40 and 45% CP; FR4 - 36, 40, 45 and 45% CP; FR5 - 40, 45, 45 and 55% CP offered every 15 days.

It is noteworthy that, in all feeding regimes, the average length and weight of the animals increased significantly in relation to managements based on a single CP level as shown in the literature (SEIXAS FILHO, 1998a and b; ALBINATI, 2000; BARBOSA et al., 2005). These results allow us to infer a possible relationship between the levels of nitrogen and the initial metabolism of tadpoles, as reported by SEIXAS FILHO et al. (2008), who verified the presence of liver cells, which had a more compact glandular arrangement, and the formation of pancreatic acini, suggesting functionality, only on the 25th day after the beginning of exogenous feeding.

We do not recommend the use of FR1 (22, 28, 32, 36% CP) because it produced the worst feed conversion during the first fifteen days, which can compromise the project financially and the animals physiologically during this phase. In the conditions of this experiment, we recommend the use of the feeding regimes FR2 (28, 32, 36 and 40% CP) and FR3 (32, 36, 40 and 45% CP) for the best performance of tadpoles and the lowest operational cost. This information can guide new experiments, improve breeding conditions, the economic value of the activity, as well as increase the number of animals sent to fattening.

Table 5 - Survival of bullfrog tadpoles fed different diets for 60 days

Feeding regimes	Biometrics (days)				
	0	15	30	45	60
FR1	100	100	90.48	90.48	86.67
FR2	100	100	86.67	84.76	80.95
FR3	100	100	93.33	90.48	86.67
FR4	100	100	86.67	86.67	83.81
FR5	100	87.62	73.33	71.43	65.71

[1]FR1 - 22, 28, 32 and 36% CP; FR2 - 28, 32, 36 and 40% CP; FR3 - 32, 36, 40 and 45% CP; FR4 - 36, 40, 45 and 45% CP; FR5 - 40, 45, 45 and 55% CP offered every 15 days.

CONCLUSION

We observed that diets with 28, 32, 36 and 40% CP and 32, 36, 40 and 45% CP were better suited to the management of bullfrogs.

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