Research, Society and Development, v. 11, n. 11, e218111133514, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i11.33514

Feeds formulated with alternative ingredients for bullfrog tadpoles (*Lithobates catesbeianus*)

Alimentos formulados com ingredientes alternativos para girinos de rã-touro *(Lithobates catesbeianus)*

Alimentos formulados con ingredientes alternativos para renacuajos de rana toro *(Lithobates catesbeianus)*

Received: 07/28/2022 | Reviewed: 08/09/2022 | Accept: 08/11/2022 | Published: 08/21/2022

Arléia Medeiros Maia
ORCID: https://orcid.org/0000-0002-3674-973X
Universidade Federal Rural do Rio de Janeiro, Brazil
E-mail: arleiamedeirosmaia2017@gmail.com
José Luiz Leonardo de Araujo Pimenta
ORCID: https://orcid.org/0000-0002-1489-2009
Universidade Estadual Paulista, Brazil
E-mail: luiz.pimenta@unesp.br
José Teixeira Seixas Filho
ORCID: https://orcid.org/0000-0002-5021-1290
Fundação Instituto de Pesca do Estado do Rio de Janeiro, Brazil
E-mail: seixasfilho@yahoo.com.br
Silvia da Conceição Reis Pereira Mello
ORCID: https://orcid.org/0000-0002-5537-3563
Empresa de Pesquisa Agropecuária do Estado do Rio de Janeiro, Brazil
E-mail: silviaqua@uol.com.br
Giselle Eler Amorim Dias
ORCID: https://orcid.org/0000-0002-4066-3824
Fundação Instituto de Pesca do Estado do Rio de Janeiro, Brazil
E-mail: giselleeler@gmail.com
Elaine da Conceição Pinto
ORCID: https://orcid.org/0000-0002-3863-6990
Fundação Instituto de Pesca do Estado do Rio de Janeiro, Brazil
E-mail: elaine.fiperg@gmail.com
Matheus Pereira dos Santos
ORCID: https://orcid.org/0000-0001-6341-1569
Universidade Federal Rural do Rio de Janeiro, Brazil
E-mail: matheusps.pereira@gmail.com
Marcelo Maia Pereira
ORCID: https://orcid.org/0000-0002-1898-2722
Fundação Instituto de Pesca do Estado do Rio de Janeiro, Brazil
E-mail: mmaiap2001@yahoo.com.br

Abstract
Several foods are available for the formulation of rations in Brazil; however, the effectiveness of a few of these foods has been evaluated in diets for bullfrog tadpoles (*Lithobates catesbeianus*). This study aimed to evaluate alternative foods (worm meal, peeled banana, peeled pumpkin, and peeled avocado) with different levels of crude protein and ether extract for the nutrition of bullfrog tadpoles to obtain the best animal performance and water quality. In a 60 days long experiment, a total of 480 bullfrog tadpoles (stage 25 of the Gosner table, 1960) with an average weight of 0.107 ± 0.004 g, were used. The experimental design was completely randomized (DIC) in a factorial scheme (2x2) with four treatments [rations with 320 g/kg crude protein (CP) and 90 g/kg ether extract (EE); 380 g/kg CP and 90 g/kg EE; T3:320 g/kg CP and 110 g/kg EE; 380 g/kg CP and 110 g/kg EE] each consisting of four replicates. To verify the normality of the data, the results of zootechnical performance, water quality, and proximate composition were subjected to the Shapiro-Wilk and Bartlett tests. Analysis of variance (ANOVA) was then performed, and the means were compared using Duncan's test at 5% of probability. The verified results of animal performance, water quality, and proximate composition were not influenced by the suggested diets. The best diet formulated based on easily acquired alternative ingredients can offer 320 g/kg CP and 90 g/kg EE to the tadpoles of bullfrog.
Keywords: Aquaculture; Food; Frog farm; Nutrition.

Resumo
Diversos alimentos estão disponíveis para a formulação de rações no Brasil; no entanto, a eficácia de alguns desses alimentos foi avaliada em dietas para girinos de rã-touro (*Lithobates catesbeianus*). Este trabalho teve como objetivo avaliar alimentos alternativos (farinha de minhoca, banana descascada, abóbora descascada e abacate descascado) com diferentes teores de proteína bruta e extrato etéreo para a nutrição de girinos de rã-touro para obter o melhor desempenho animal e qualidade da água. Em um experimento de 60 dias, foram utilizados um total de 480 girinos de rã-touro (estágio 25 da tabela de Gosner, 1960) com peso médio de 0,107 ± 0,004 g. O delineamento experimental foi inteiramente casualizado (DIC) em esquema fatorial (2x2) com quatro tratamentos [rações com 320 g/kg de proteína bruta (PB) e 90 g/kg de extrato etéreo (EE); 380 g/kg CP e 90 g/kg EE; T3: 320 g/kg CP e 110 g/kg EE; 380 g/kg CP e 110 g/kg EE] cada um consistindo em quatro réplicas. Para verificar a normalidade dos dados, os resultados de desempenho zootécnico, qualidade da água e composição centesimal foram submetidos aos testes de Shapiro-Wilk e Bartlett. Foi então realizada a análise de variância (ANOVA) e as médias comparadas pelo teste de Duncan a 5% de probabilidade. Os resultados verificados de desempenho animal, qualidade da água e composição centesimal não foram influenciados pelas dietas sugeridas. A melhor dieta formulada com base em ingredientes alternativos de fácil aquisição pode oferecer 320 g/kg de PB e 90 g/kg de EE aos girinos de rã-touro.
Palavras-chave: Alimento; Aquicultura; Nutrição; Ranicultura.

Resumen
Varios alimentos están disponibles para la formulación de raciones en Brasil; sin embargo, se ha evaluado la efectividad de algunos de estos alimentos en dietas para renacuajos de rana toro (*Lithobates catesbeianus*). Este estudio tuvo como objetivo evaluar alimentos alternativos (harina de gusano, plátano pelado, calabaza pelada y aguacate pelado) con diferentes niveles de proteína cruda y extracto etéreo para la nutrición de renacuajos de rana toro para obtener el mejor rendimiento animal y calidad del agua. En un experimento de 60 días de duración, se utilizaron un total de 480 renacuajos de rana toro (etapa 25 de la tabla de Gosner, 1960) con un peso promedio de 0,107 ± 0,004 g. El diseño experimental fue completamente al azar (DIC) en esquema factorial (2x2) con cuatro tratamientos [raciones con 320 g/kg de proteína cruda (PB) y 90 g/kg de extracto etéreo (EE); 380 g/kg PB y 90 g/kg EE; T3: 320 g/kg CP y 110 g/kg EE; 380 g/kg CP y 110 g/kg EE], cada uno de los cuales consta de cuatro repeticiones. Para verificar la normalidad de los datos, los resultados de desempeño zootécnico, calidad del agua y composición próxima fueron sometidos a las pruebas de Shapiro-Wilk y Bartlett. Luego se realizó el análisis de varianza (ANOVA) y las medias se compararon usando la prueba de Duncan al 5% de probabilidad. Los resultados verificados de desempeño animal, calidad del agua y composición próxima no fueron influenciados por las dietas sugeridas. La mejor dieta formulada a base de ingredientes alternativos de fácil adquisición puede ofrecer 320 g/kg de PC y 90 g/kg de EE a los renacuajos de rana toro.
Palabras clave: Acuicultura; Alimento; Granja de ranas; Nutrición.

1. Introduction

Frog farming is divided into two well-defined phases: aquatic and terrestrial farming. In the aquatic phase, tadpoles are omnivorous and feed on mash diets (Mansano et al., 2014) with a lower protein content than that present in the diet of frogs after metamorphosis. The frogs, in turn, are carnivores that feed on pelleted or extruded diets (Pereira et al., 2015).

Ingredients used for freshwater aquaculture are divided into 3 categories in Brazil: energy foods, protein foods of animal origin, and protein foods of plant origin (Pezzato et al., 2010). Few of these foods have been evaluated for their digestibility for bullfrog tadpoles (*Lithobates catesbeianus*), such as fish meal, soy flour, cornmeal, corn starch, and soybean oil (Albinatti et al., 2000); rice bran, wheat bran, blood meal, and poultry viscera meal (Secco et al., 2005); and acid silage from tilapia filleting residues (Oliveira et al., 2008).

New foods must be tested for tadpoles if they have the following characteristics: easy availability, nutrient richness, adequate mineral content, and highly digestible vitamin content. Moreover, they must contribute to the economic and environmental sustainability of agricultural activity (Seixas Filho et al., 2022).

Among the alternate foods, earthworm flour, pulps of banana, pumpkin, and avocado are easily found in the different centers of food distribution and commerce in Brazil.

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i11.33514

The objective of this study was to evaluate diets containing alternative foods with different levels of crude protein and ether extract for the nutrition of bullfrog tadpoles, with the aim of obtaining the best animal performance and water quality.

2. Methodology

The experiments, conducted at the Fisheries Institute Foundation of the State of Rio de Janeiro (FIPERJ), were approved by the Ethics Committee on the Use of Animals (CEUA) under FIPERJ No. 002-2018, and lasted for 60 days.

A total of 480 bullfrog tadpoles in stage 25 (Gosner, 1960) were used. Based on the same spawning, with an average weight of 0.107 ± 0.004 g, the animals were packed in 60% volume polypropylene boxes, with a capacity of 50 L, at a density of 1 tadpole/L, to compose an experimental unit.

A total of 16 polypropylene boxes was placed side by side on benches; aeration was maintained with a 3/16 inch diameter hose with a porous stone at its end. A water recirculation system (Seixas Filho et al., 2013) was used for 200% water renewal in 24 h.

The experimental units were cleaned daily to remove leftover feed and feces. A few water quality parameters were analyzed. The water temperature was measured with a thermometer. The levels of total ammonia (NH_4 and NH_3), toxic ammonia (NH_3), and the pH of the water were measured using a commercial colorimetric kit, and dissolved oxygen was measured using a digital oximeter.

The experimental design was completely randomized (DIC) in a factorial scheme (2x2) with four treatments each having four replicates, in a total of 16 experimental units. The treatments were conducted in a factorial scheme with two levels of crude proteins (320 and 380 g/kg) and two levels of ether extract (9 and 11 g/kg) (Table 1).

Table 1. Experimental diets balanced with different levels of crude protein (CP) and ether extract (EE) for bullfrog tadpoles (*Lithobates catesbeianus*).

	Treatements			
	380 PB / 90 EE	**380 PB / 110 EE**	**320 PB / 90 EE**	**320 PB / 110 EE**
Ingredient	**Quantity (%)**			
Salmon fishmeal	34.38	34.38	29.38	29.38
Worm meal	25.00	25.00	20.00	20.00
Peeled banana	15.00	15.00	25.00	25.00
Peeled Pumpkin	16.00	12.00	16.00	12.00
Peeled Avocado	9.00	13.00	9.00	13.00
Mineral/Vitamin[1]	0.60	0.60	0.60	0.60
BHT	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00
	Composition			
Crude Protein (g/Kg)	383.6	380.6	327.0	324.0
Gross Energy (Kcal/Kg)	4,249.68	4,385.98	4,208.00	4,344.72
Ethereal extract (g/Kg)	99.1	116.9	91.8	109.7

[1]Premix contains: Vitamin A (IU/kg): 600,000, Vitamin D3 (IU/kg): 600,000, Vitamin E (mg/kg): 12,000, Vitamin K3 (mg/kg): 631, Thiamine B1 (mg/kg): 1,176, Riboflavin B2 (mg/kg): 1,536, Pyridoxine B6 (mg/kg): 1,274, Vitamin B12 (mcg/kg): 4,000, Niacin (mg/kg): 19,800, Pantothenic Acid B3 (mg/kg): 3,920, Folic Acid (mg/kg): 192, Biotin (mg/kg): 20, Vitamin C (mg/kg): 40,250, Choline (mg/kg): 30,000, Moisture (%): 2.0, Ash (%): 71.6442, Magnesium (%): 0.0085, Sulfur (%): 1.1589, Iron (mg/kg): 25,714, Copper (mg/kg): 1,960, Manganese (mg/kg): 13,345, Zinc (mg/kg): 30,000, Iodine (mg/kg): 939, Selenium (mg/kg): 30. Fonte: Autores (2022).

The statistical model adopted was a factorial experiment with two factors: the α factor (crude protein) with i levels (two) and the β factor (ethereal extract) with j levels (two), installed according to the DIC, with k repetitions (four), which are represented as follows:

$$Y_{ijk} = m + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

Ingredients such as flours made of pulps of banana, pumpkin, and avocado were obtained under an experimental process that is under patent secrecy (Registration Institution: INPI - National Institute of Industrial Property, filing date: 10/10/2019). All ingredients were analyzed for their centesimal composition (Table 2). Diets were prepared using a pelleted diet protocol (Fracalossi et al., 2012) and then ground, and sieved.

Table 2. Composition of three experimental alternative ingredients used in the proposed diets for bullfrog tadpoles (*Lithobates catesbeianus*).

Composition	Peeled banana	Peeled Pumpkin	Peeled Avocado
Mouisture (g/kg)	186.9	55.5	109.2
Protein (g/kg)	34.4	54.9	130.1
Fat (g/kg)	4.4	493.5	47.7
Ash (g/kg)	25.0	45.1	56.1
GE (kcal/kg)	3,714.02	7,177.04	3,769.50

Fonte: Autores (2022).

For all ingredients and diets were analyzed for the dry matter using an oven at 105°C (Silva and Queiroz, 2006), for crude protein following the Kjeldhal method (AOAC, 1984), for the ether extract using the method of Folch (1957), for raw energy using a calorimetric bomb (Silva and Queiroz, 2006), and for ash by incineration in a muffle furnace at 600 (Silva and Queiroz, 2006).

The animals received the diets (8% of the live weight, distributed four times a day) in mash form (Seixas Filho et al, 1998). The amount supplied was recorded to calculate the consumption of diets.

Biometrics was performed at 0, 15, 30, 45, and 60 days of the experiment; the individual weight (g) of the tadpoles was recorded on a digital scale (0.001 g).

At the end of the experiment, 30 g of live weight of tadpoles from each repetition was sampled for analysis of the proximate compositions (water, protein, ash, and fat).

The sampled animals were stunned by thermonarcosis, by placing them in containers filled with 50% water and 50% ice. subsequently, the animals were stored frozen until the biological material was processed.

Frozen tadpole samples (intact body) were homogenized in a processor, placed in identified containers, and kept in a freezer at -10 °C on 60 experimental days.

The dry matter was determined by keeping the sample in an oven at 105°C (Silva and Queiroz, 2006). Crude protein analysis was performed using the Kjeldahl method (AOAC, 1984), crude lipid (Ethereal extract) was assessed using the Folch method (1957), and ash was prepared in a muffle furnace at 600 °C by incineration (Silva and Queiroz, 2006).

The results found for the evaluated parameters of zootechnical performance [weight, zootechnical indices of Weight Gain (WG), Apparent Feed Conversion (AFC), and Specific Growth Rate (SGR)], water quality, and proximate composition were submitted to the Shapiro tests (Wilks and Barlett) to verify the normality and homoscedasticity of the data. Analysis of variance (ANOVA) was performed, and in cases of differences between the means, they were compared by Duncan's test at the 5% probability level. All statistical procedures were performed with the aid of a statistical program (SAS, 2008).

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i11.33514

3. Results

The mean values of the observed physicochemical parameters of water were within the standards required for bullfrog tadpoles (Table 3).

Table 3. Mean values and standard deviations for temperature (T °C H2O), dissolved oxygen (OD) NH_3, NH_4 and pH of the water of the experimental units that conditioned the bullfrog tadpoles (*Lithobates catesbeianus*) with different levels of protein crude (CP) and ethereal extract (EE).

Diets		**OD (mg/L)**	**T °C H_2O**	**NH_3 (mg/L)**	**NH_4 (mg/L)**	**pH**
CP (g/Kg)	EE (g/Kg)					
320	90	5.37±0.22	25.2±0.50	0.0009±0.0004	0.39±0.25	5.92±0.48
320	110	5.22±0.28	25.3±0.46	0.0009±0.0004	0.34±0.22	5.79±0.67
380	90	5.82±0.26	25.1±0.46	0.0009±0.0004	0.34±0.22	6.06±0.63
380	110	5.11±0.36	25.0±0,.46	0.0009±0.0004	0.34±0.22	5.63±0.53

Fonte: Autores (2022).

During the experimental period, no significant differences (P>0.05) were observed in the weights of bullfrog tadpoles fed with different levels of crude protein (320, 380 g/kg) and in those fed with different levels of ether extract (90, 110 g/kg); and, the increase in the weight were not influenced by the diets during the entire experimental period (Table 4).

Table 4. Mean and standard error of weight (g) of bullfrog tadpoles (*Lithobates catesbeianus*) fed diets with different levels of crude protein (CP) and ether extract (EE).

Diets		**Weight (g)**				
CP (g/Kg)	**EE (g/Kg)**	**1 (day)**	**15 (days)**	**30 (days)**	**45 (days)**	**60 (days)**
320	90	0.104 ± 0.008	0.438 ± 0.095	1.555 ± 0.256	1.989 ± 0.183	3.088 ± 0.499
320	110	0.149 ± 0.023	0.840 ± 0.105	1.664 ± 0.304	2.405 ± 0.183	3.047 ± 0.198
380	90	0.114 ± 0.003	0.423 ± 0.083	1.247 ± 0.245	1.747 ± 0.133	2.569 ± 0.299
380	110	0.148 ± 0.016	0.676 ± 0.181	1.600 ± 0.150	2.058 ± 0.083	2.859 ± 0.142
P-Value		0.7291	0.5524	0.6264	0.7338	0.6100
CP (g/Kg)						
320		0.126 ± 0.014	0.639 ± 0.100	1.609 ± 0.185	2.197 ± 0.143	3.068 ± 0.249
380		0.131 ± 0.010	0.549 ± 0.104	1.424 ± 0.149	1.902 ± 0.093	2.714 ± 0.163
P-Value		0.7855	0.4770	0.4632	0.0752	0.2841
EE (g/Kg)						
90		0.109 ± 0.005	0.430 ± 0.058	1.401 ± 0.174	1.868 ± 0.104	2.829 ± 0.286
110		0.148 ± 0.013	0.758 ± 0.102	1.632 ± 0.157	2.232 ± 0.114	2.953 ± 0.118
P-Value		0.0826	0.0198	0.3640	0.0333	0.7008

*P-value < 0.05, means differ by analysis of variance. Fonte: Autores (2022).

In general, the average body weight was not influenced (P>0.05) by crude protein (CP) and ether extract (EE) content in the diet. The balance between the CP and EE content in bullfrog tadpole diets improved the efficiency of these animals.

The zootechnical indices of Weight Gain (WG), Apparent Feed Conversion (AFC), and Specific Growth Rate (SGR) of bullfrog tadpoles did not show significant differences (P>0.05) between animals fed diets containing different CP and EE levels (Table 5).

Table 5. Mean and standard error of total weight gain (WG), apparent feed conversion (AFC) and specific growth rate (SGR) of bullfrog tadpoles (*Lithobates catesbeianus*) fed diets with different levels of crude protein (CP) and ether extract (EE).

Diets		**Zootechnical Indices**		
CP (g/Kg)	**EE (g/Kg)**	**WG (g)**	**AFC (g/g)**	**SGR (%/dia)**
320	90	2.984 ± 0.493	1.715 ± 0.485	5.588 ± 0.227
320	110	2.898 ± 0.202	1.218 ± 0.030	5.072 ± 0.263
380	90	2.456 ± 0.297	1.893 ± 0.252	5.163 ± 0.173
380	110	2.711 ± 0.128	1.465 ± 0.112	4.959 ± 0.115
P-Value		0.5945	0.8879	0.4561
CP (g/Kg)				
320		2.941 ± 0.247	1.431 ± 0.209	5.330 ± 0.188
380		2.583 ± 0.157	1.679 ± 0.151	5.061 ± 0.104
P-Value		0.2736	0.3951	0.2083
EE (g/Kg)				
90		2.720 ± 0.285	1.817 ± 0.230	5.376 ± 0.155
110		2.805 ± 0.116	1.342 ± 0.071	5.016 ± 0.134
P-Value		0.7909	0.0797	0.1009

*P-value < 0.05, means differ by analysis of variance. Fonte: Autores (2022).

The body composition (moisture, ash, protein, and ether extract) of bullfrog tadpoles was not affected (P>0.05) by crude protein and ether extract levels during the study period (Table 6).

Table 6. Mean and standard error of body composition (Moisture, Ash, Protein and Fat) of bullfrog tadpoles (*Lithobates catesbeianus*) fed diets with different levels of crude protein (CP) and ether extract (EE).

Diets		**Body composition**			
CP (g/Kg)	**EE (g/Kg)**	**Moisture (g)**	**Ash (g)**	**Protein (g)**	**Fat (g)**
320	90	2.128 ± 0.344	0.165 ± 0.027	0.651 ± 0.105	0.143 ± 0.023
320	110	1.945 ± 0.127	0.184 ± 0.012	0.741 ± 0.048	0.177 ± 0.011
380	90	1.663 ± 0.193	0.162 ± 0.022	0.619 ± 0.082	0.125 ± 0.014
380	110	1.813 ± 0.090	0.184 ± 0.009	0.688 ± 0.043	0.174 ± 0.009
P-Value		0.4474	0.9442	0.8906	0.6297
CP (g/Kg)					
320		2.037 ± 0.173	0.175 ± 0.014	0.696±0.056	0.160 ± 0.013
380		1.738 ± 0.102	0.172 ± 0.012	0.654±0.043	0.149 ±0.012
P-Value		0.1839	0.9272	0.5736	0.4950
EE (g/Kg)					
90		1.896 ± 0.202	0.163 ± 0.016	0.635 ± 0.062	0.134 ± 0.013
110		1.879 ± 0.076	0.184 ± 0.007	0.714 ± 0.029	0.175 ± 0.007
P-Value		0.9375	0.2953	0.3007	0.0203

*P-value < 0.05, means differ by analysis of variance. Fonte: Autores (2022).

4. Discussion

The average dissolved oxygen (DO) observed in the four treatment sets (of 5.37±0.22; 5.22±0.28; 5.82±0.26 and 5.11±0.36) was nearly parallel to that reported by a previous study on the requirement of digestible protein for tadpoles (Pinto et al., 2015).

The water temperature remained constant in all treatments. Since water directly influences the metabolism of ectotherms such as tadpoles, promoting greater performance under ideal thermal conditions (Castro and Pinto, 2000), the minimum range of temperature variation of rearing water can be considered satisfactory and does not influence the development of tadpoles.

Ammonia (NH_3) concentrations were checked periodically, and it remained <1 mg/L (0.0009±0.0004) in all treatments evaluated. This concentration was maintained in the experimental units through daily cleaning, i.e., removing leftover feed and feces from the bottom. The NH_3 and NH_4 contents are crucial due to their toxicity that depends on the pH and temperature of the culture water (Thuller et al., 2017).

The average pH values obtained throughout the study showed little variation between treatments. A pH range between 6.0 and 7.0 is considered ideal for bullfrog tadpoles (Mansano et al., 2016).

During the first 45 days of life, body weight of tadpoles increases in an accelerated and considerable way and then in a more continuous and regular way. It is closely related to the metamorphic development of these animals (Carmona-Osalde et al., 1996).

The present study demonstrated that the protein content in the diet affects the rate of development in tadpoles, and the metamorphic climax could only be reached when the tadpoles meet their nutritional needs, which would allow them to transform into a new and completely different animal. Inadequate diets prolonged the larval stage of *Pelophylax perezi* (Martinez et al., 1994). In this experiment, the results revealed a balance in the performance of animals with different rations, thus revealing a flexibility of bullfrog tadpoles for their food and nutrition.

In addition, the food materials used in the study were potent source of better quality protein, since the ingredients were not grains with a greater amount of fiber in the cell wall; instead they were of vegetable origin and contained cell wall with less amount of lignin, hemicellulose, and other fibers. It facilitated their absorption by the digestive tract of the tadpole.

The levels of crude protein and ethereal extract in this study met the nutrient needs of bullfrog tadpoles. Further studies are needed to assess lower levels of these nutrients to find a minimum requirement for this phase of rearing these animals with the same or similar ingredients in the rations. The minimum amount of protein in the diet is important to ensure the growth and development of tadpoles; moreover, it influences immunity against diseases (Seixas Filho et al., 2012).

Diets with less digestible protein content have lower market prices (Seixas Filho et al., 2010) and can reduce the impact of pollution caused by production residues (Amirkolaie, 2011; Bosma and Verdegem, 2011).

The data regarding apparent feed conversion (AFC) showed no difference between the treatments evaluated, and the values found in another study for bullfrog tadpoles ranged from 0.62 to 1.68 (Lima et al., 2003), ideal in systems where feeding is based on ration only, the closer to 1 the better.

The experimental diets in this study met the nutritional requirements of bullfrog tadpoles, which can be confirmed by the total weight gain (g/day). As demonstrated by the percentage of survival, a large protein content is not required in the ration for satisfactory development of tadpoles. Similarly, the specific growth rate was not affected by the diet, showing satisfactory results.

Extrinsic factors, such as nutritional management, fasting period, and pre-slaughter management can cause changes in body composition (percentage of fat, protein, minerals, and vitamins), as excessive stress occurs in animals at these stages (Alves et al., 2016). Stress that may occur at the time of sacrifice result in a decrease or loss of body nutrients.

The growth of an animal is directly related to its weight gain, which is directly related to the retention of water, protein, fat, and minerals in the body tissue, and it can vary from organism to organism (Mazzini et al., 2003).

In a study of nutrient deposition in the carcasses of bullfrog tadpoles fed with a commercial ration of 550 g/kg CP, 6.97% crude protein was found in the carcasses of the animals (Mansano et al., 2013). In this study, 21.00% of crude protein was found in the carcasses of bullfrog tadpoles fed rations with 320 g/kg of CP and 90 g/kg of EE. Hence, presence of the alternative ingredients in the diet instead of the traditional ones altered nutrient deposition.

The partial replacement of animal-derived flour (salmon flour) and total vegetable-derived flour, such as soybean meal and corn bran, with alternative plant-based ingredients such as avocado, pumpkin, and banana, can be a good strategy for cost reduction and production with unaltered weight gain of bullfrog tadpoles (Seixas Filho et al., 2022).

In addition, this substitution of ingredients did not affect the quality in the zootechnical performance of the animals, even more when dealing with a minutely critical phase such as that of tadpoles, where food is fundamental for the health, proper development, and metamorphosis of the animal (Mansano et al., 2016). In turn, a reduction in the cost of the diet and increased production could enhance profits for frog breeders significantly and make the strategy viable.

Since commercial fish rations cause damage to the health, mainly to the liver, of bullfrog tadpoles, future studies are required to analyze the histological quality of the liver of tadpoles (Seixas Filho et al., 2008).

Avocado (*Persea americana*) plant contains high levels of oleic monounsaturated fatty acid, high fiber content that serve as an energy reserve, and vitamins B1, B2, C, D, and E. Avocado pulp is rich in iron, calcium, phosphorus, and lipids, which are rare in other fruits (Salgado et al., 2005).

Pumpkin (*Cucurbita maxima*) is a fleshy and juicy vegetable coated with skin that can vary from semi-soft to very hard. It is rich in pro-vitamin A, zinc, phosphorus, calcium, iron, and carotenoids. The major carotenoids present in pumpkin are α-carotene, β-carotene, and lutein (Naves et al., 2010).

Banana (*Musa* sp.) is a highly energetic food with approximately 100 kcal/100 g of pulp. Among its properties, vitamins C, A, B1, B2, D, and E can be highlighted in decreasing percentages; minerals such as potassium, phosphorus, calcium, iron, and magnesium, and fibers can help in the passage of chyme through the digestive tract. Although approximately 20% of carbohydrates are easily assimilated by the body, it lacks proteins and lipids (Borges, 2009).

It is important to emphasize that tadpoles have amylases in their digestive tract (Seixas Filho et al., 2010), thus allowing good use of the components studied in this work.

5. Conclusion

The best feed for bullfrog tadpoles under the experimental conditions proposed in this study contained 32% CP and 9 kcal/kg EE. Easily available alternative ingredients of plant origin, such as avocado, pumpkin, and banana, can be adopted in bullfrog tadpole diets.

Acknowledgments

We would like to thank Editage (www.editage.com) for English language editing.

References

Albinati, R. C. B., Lima, S. L., Tafuri, M. L. & Donzele, J. L. (2000). Digestibilidade aparente de dois alimentos protéicos e três energéticos para girinos de rã-touro (*Rana catesbeiana*, Shaw, 1802). *Revista Brasileira de Zootecnia*, 29(6):2151-2156.

Alves, A. R., Figueiredo Júnior, J. P., Santana, M. H. M., Andrade, M.V.M., Lima, J.B.A.; Pinto, L.S.P. & Ribeiro, L.M. (2016). Efeito do estresse sobre a qualidade de produtos de origem animal. *Pubvet*, 10(6):448-459.

Amirkolaie, A. K. (2011). Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. *Reviews in Aquaculture*, 3:19-26.

AOAC - Association of Official Analytical Chemists. (1984). *Official methods of analysis*. 14. ed. Arlington Virginia, p.1141.

Borges, A. M., Pereira, J. & Lucena, E. M. P. (2009). Caracterização da farinha de banana verde. *Ciências e tecnologia de alimentos*. 29(2):333-339.

Bosma, R. H. & Verdegem, M. C. J. (2011). Sustainable aquaculture in ponds: Principles, practices and limits. *Livestock Science*, 139:58-68.

Carmona-Osalde, C., Olivera-Novoa, M. A., Rodríguez-Serna, M. & Flores-Nava, A. (1996). Estimation of the protein requirement for bullfrog (*Lithobates Catesbeianus*) tadpoles, and its effect on metamorphosis ratio. *Aquaculture*, 141:223-231.

Castro, J. C. & Pinto, A. T. (2000). Qualidade da água em tanques de girinos de rã-touro, (*Rana catesbeiana* Shaw, 1802), cultivados em diferentes densidades de estocagem. *Revista Brasileira de Zootecnia*, 29:1903-1911.

Fracalossi, D. M., Rodrigues, A. P. O., Silva, T. S. C. & Cyrino, J. E. P. (2012). Técnicas experimentais em nutrição de peixes. In: *Nutriaqua - Nutrição e alimentação de espécies de interesse para a aquicultura brasileira*, 37-64. Florianópolis: Copiart.

Folch, J., Lees, M. S. & Tanley-sloane, G. H. (1957). A simple method for the isolation purification of total lipids from animal tissues. *Journal Biology Chemistry*, 226:497-507.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16:183-190.

Lima, S. L., Casali, A. P. & Agostinho, C. A. (2003). Desempenho zootécnico e tabela de alimentação de girinos de rã-touro (*Rana catesbeiana)* criados no sistema anfigranja. *Revista Brasileira de Zootecnia*, 32:512-518.

Mansano, C. F. M., Stéfani, M. V., Pereira, M. M. & Macente, B. I. (2013). Deposição de nutrientes na carcaça de girinos de rã-touro. *Pesquisa Agropecuária Brasileira*, 48(8):885-891.

Mansano, C. F. M., Stéfani M. V., Pereira M. M., Nascimento T. S. R. & Macente B. I. (2014). Morphometric growth characteristics and body composition of bullfrog tadpoles in captivity. *Semina. Ciênc. Agrárias,* 35:1425-1438.

Mansano, C. F .M., Pereira, M. M., Macente, B. I., Makina, L. C., Jacintho, A. P. P., Nakaghi, L. S. O. & Stéfani, M. V. (2016). Diferentes níveis proteicos na dieta de girinos de rã-touro (*Lithobates catesbeianus)* e seus efeitos sobre o tecido hepático. *Pesquisa Veterinária Brasileira*, 6(6):1-10.

Mazzini, A. R. A., Muniz, J. A., Aquino, L. H. & Silva, F. F. (2003). Análise da curva de crescimento de machos hereford. *Ciência e Agrotecnologia*, 27(5):1105-1112.

Naves, L. P., Corrêa, A. D., Abreu, C. M. P. & Santos, C. D. (2010). Nutrientes e propriedades funcionais em sementes de abóbora (*Cucurbita maxima*) submetidas a diferentes processamentos. *Ciência e Tecnologia de Alimentos*, 30:185-190.

Oliveira, M. M., Pimenta, M. E. S. G., Camargo, A. C. S, Pimenta, C. J. & Logato, P. V. R. (2008). Silagem ácida de resíduos da filetagem de tilápias para girinos de rã-touro (*Rana catesbeiana* Shaw,1802): Digestibilidade e desempenho. *Ciência e Agrotecnologia*, 32:618-625.

Pereira, M. M., Mansano, C. F. M., Peruzzi, N. J. & Stefani, M. V. (2015). Nutrient deposition in bullfrogs during the fattening phase. *Boletim do Instituto de Pesca*, 41:305 – 318.

Pezzato, E. L., Barros, M. M. & Furuya, W. M. (2010). Valor nutritivo dos alimentos utilizados na formulação de rações para peixes tropicais. *Revista Brasileira de Zootecnia*, 38:43-51.

Pinto D. F. H., Mansano C. F. M., De Stéfani M. V. & Pereira M. M. (2015). Optimal digestible protein level for bullfrog tadpoles. *Aquaculture*. 440:2-16.

Salgado, J. M. (2005). Alimentos Inteligentes Saiba como Obter Mais Saúde por Meio da Alimentação. *Prestígio*, p.27-29.

Secco, E. M., Stéfani, M. V. & Vidotti, R. M. (2005). Apparent digestibility of different ingredients in diets for bullfrog (*Lithobates Catesbeianus)* tadpoles. *Journal of the World Aquaculture Society*, 36:135-140.

Seixas Filho, J. T., Oliveira, M. G. A., Navarro, R. D., Garcia, S. L. R., Moura, G. S. & Ribeiro Filho, O. P. (1998). Atividades enzimáticas de girinos de rã-touro submetidos a rações com níveis de proteína. *Archivos de Zootecnia*, 60:1161-1170.

Seixas Filho, J. T., Hipólito, M., Carvalho, V. F., Martins, A. M. C. R. P. F., Silva, L. N. & Castagna, A. A. (2008). Alterações histopatológicas em girinos de rã-touro alimentados com rações comercias de diferentes níveis protéicos. *Revista Brasileira de Zootecnia,* 37:2085-2089.

Seixas Filho, J. T., Navarro, R. D., Silva, L. N., Garcia, S.L.R. & Hipólito, M. (2010). Desempenho de girinos de rã- touro alimentados com ração comercial contendo diferentes concentrações de proteína bruta. *Revista Brasileira de Ciências Agrárias*, 5(3):428-433.

Seixas-Filho, J. T., Mello, S. C. R. P., Veiga, R. C. A., Miranda, R. G. B. & Santos, C A. N. (2010). Efeito da granulometria da ração sobre o desempenho de girinos de (*Lithobates Catesbeianus*). *Revista Brasileira de Zootecnia*, 27:224-230.

Seixas Filho, J. T., Navarro, R. D., Pereira, M. M., Mello, S. C. R. P., Lanna, E. A. T. & Lima, J. L. P. (2012). Desempenho zootécnico de girinos de rã-touro com diferentes níveis de proteína e energia digestíveis. *Revista Brasileira de Saúde e Produção Animal*, 13(4):1112-1120.

Seixas Filho, J. T., Mello, S. C. R. P., Freitas Junior, W. G. & Silva, D. F. F. E. (2013). *Implantação de sistema de filtragem biológica para reuso de água na produção de girinos de rã-touro*. Rio de Janeiro: Unisuam Publicações, 1:14.

Seixas Filho, J. T., Mello, D .S., Mello, S. C. R. P., Pereira, M. M., Dias, G. E. A., Pinto, E. C. & Silva, W .N. (2022). Performance of bullfrog tadpoles (*Lithobates catesbeianus*) fed balanced diets using alternative energy ingredients containing vegetable mesocarp. *Revista Brasileira de Saúde e Produção Animal*, Salvador, 23(01-14):e2122312021.

Silva, D. J. & Queiroz, A. C. (2002). *Análise de alimentos (métodos químicos e biológicos).* (3a ed.) Editora UFV. 235p.

Thuller, M. A. O., Rego, R. S., Alves, V. C., Seixas Filho., J. T. & Mello, S. C. R. P. (2017). Qualidade da água e reuso da água. In: Seixas Filho, J. T.; Pereira, M. M.; Mello, S. C. R. P. *Manual de Ranicultura para o Produtor*. HP Editora e Comunicações, 155 p.