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Performance of bullfrog tadpoles (*Lithobates catesbeianus*) fed balanced diets using alternative energy ingredients containing vegetable mesocarp

Desempenho de girinos de rã-touro (Lithobates catesbeianus) alimentados com dietas balanceadas com ingredientes de energia alternativa utilizando mesocarpo vegetal

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ABSTRACT

The objective of this study was to evaluate the performance and metamorphosis of bullfrog tadpoles (*Lithobates catesbeianus*) fed balanced diets, wherein corn flour was replaced with banana, avocado, and pumpkin meal (all made without the epicarp). Using a completely randomized design, the animals were stored in tanks with a capacity of 30 L in a water recirculation system at a density of 1 tadpole/L. Through biweekly biometric measurements, the weight, standard, and total length were evaluated to determine metamorphic development, weight gain, consumption, feed conversion, specific growth rate, survival, physical-chemical parameters of water, and the percentage carcass composition (only at the end). Tadpoles fed rations balanced with pumpkin and banana flour performed the best, followed by those fed avocado meal and those fed the control ration ($p < 0.05$). It can be concluded that growth performance is improved in animals fed diets containing 10% pumpkin and banana meal instead of corn, and tadpoles that received the avocado meal had the same performance as the conventional diets. Research should be carried out to assess other avenues for the replacement of ingredients in rations for bullfrog tadpoles.
Keywords: carbohydrate, formulation, lipid, protein

RESUMO

O objetivo deste trabalho foi avaliar o desempenho e a metamorfose de girinos de rã-touro (*Lithobates catesbeianus*) alimentados com dietas balanceadas com farinha de banana, abacate e abóbora, todas feitas sem epicarpo, em substituição à farinha de milho. Utilizando um delineamento inteiramente casualizado, os animais foram colocados em tanques com capacidade de 30 litros em sistema de recirculação de água com densidade de 1 girino / L. Por meio de biometria quinzenal foram avaliados peso, comprimento padrão e total, quanto ao desenvolvimento metamórfico, ganho em peso, consumo, conversão alimentar, taxa de crescimento específico, sobrevivência, parâmetros físico-químicos da água e composição centesimal da carcaça (somente no final). Os resultados mostraram melhor desempenho para girinos alimentados com ração balanceada com farinha de abóbora e banana, seguidos daqueles alimentados com farelo de abacate e alimentados com ração controle ($p < 0,05$). Os animais alimentados com as duas últimas rações não diferiram ($p > 0,05$). Pode-se concluir que houve melhor desempenho nos animais alimentados com dietas contendo 10% de abóbora e farelo de banana ao invés de milho, e aqueles que receberam farelo de abacate com o mesmo desempenho das dietas tradicionais. Pesquisas devem ser realizadas para avaliar outros níveis de reposição desses nutrientes por girinos de rã-touro.
Palavras-chave: carboidrato, formulação, lipídio, proteína

INTRODUCTION

Many of the ingredients that make up the rations used in aquaculture in Brazil are still not studied in relation to the intense peculiarities of the digestive physiology of aquatic organisms bred in captivity, especially in frog farming (Seixas Filho et al., 2011).

Although a satisfactory number of feedstuffs are available in Brazil for dietary formulations, only few of these ingredients have been evaluated for their digestibility in bullfrog tadpoles (Albinati et al., 2000; Secco et al., 2005; Oliveira et al., 2008). Digestibility values are available for only 10 feed ingredients used for bullfrog tadpoles, whereas there are values for 40 feedstuffs used in tilapia diets, according to the Brazilian Nutrition Tables (Furuya, 2010).

The digestive tract in the tadpole phase, unlike fingerlings, is in provisional formation and includes the pancreas and liver from the beginning of exogenous feeding (Seixas Filho et al., 2008a) when the animal is in the 25th larval stage, according to Gosner (1960). The tract is however only functional approximately 30 days later (Seixas Filho et al., 2011). This makes it difficult to absorb some of the nutrients present in the feed ingredients. This is aggravated when nutrients come from plant seeds with a high concentration of hemicellulose and lignin in the cell wall, such as corn and soybean. In contrast, cells from the ovarian region of the plants have lower fiber in the cell walls to facilitate expansion during fruit formation and are postulated to be utilized better. Among the feed ingredients that could be used as alternatives for bullfrog tadpoles, three are potentially interesting due to the structure of their cell wall, namely the meals of banana (*Musa* ssp.), pumpkin (*Cucurbita maxima*), and avocado (*Persea americana*) (Wegner and Belik, 2012; Silva et al., 2015).

In contrast to corn, which is the seed of the vegetable, banana, squash, and avocado are formed from a structure of the plant tissue called the “pseudofruit” in the case of the banana, and the “mesocarp” in the case of pumpkin and avocado; all have thinner cell walls than corn grain. The differences in the cellular structures permit the hypothesis that there is a better digestibility of these ingredients in the ration for bullfrog tadpoles.

The objective of the present work was to evaluate the performance of bullfrog tadpoles fed balanced rations using flours made from the mesocarp of banana, avocado, and pumpkins to replace 77% of the total amount of corn.

MATERIAL AND METHODS

Experimental conditions and animals

The experiment, which lasted 60 days, was conducted in the city of Rio de Janeiro, State of Rio de Janeiro, of Brazil (22º 59′ latitude and 43º 35′ 22.4″ longitude). Research on animals was conducted according to the Institutional Committee on Animal Use (number of protocol: 002/2018).

A total of 480 bullfrog tadpoles in Gosner stage 25 (1960), with a mean weight of 0.107 ± 0.004 g, originating from the same spawn, were used. The animals were housed in 60% of the volume of 50 L polypropylene tanks at a density of 1 tadpole/L, which corresponded to one experimental unit. The polypropylene tanks were placed side by side on benches in 16 units aerated with a 3/16″ tube containing a porous stone at the terminus. The water exchange was 57 times the volume of 30 L in 24 h.

The experimental units were siphoned daily and the water temperature was measured with a thermometer. Water levels of NH_4 and NH_3 and water pH

were obtained daily using a commercial kit for the control of water quality in the tanks (LABTEST®).

Experimental design and diets

The experimental design was completely randomized and consisted of four treatments, with four replications per treatment, totaling 16 experimental units. Treatments corresponded to the substitution of 10% of the corn for peeled banana, avocado, and pumpkin meals (Table 1), plus a corn-based control. The diets (Table 2) were formulated according to the digestibility values reported by Secco et al. (2005).

The animals received the diets in meal form (Seixas Filho et al., 1998), corresponding to 8% of their live weight. The diet was supplied four times daily, and the amounts provided were recorded for the calculation of feed intake.

Table 1. Composition of peeled banana, pumpkin, and avocado meals substituting 10% of the corn in diets for bullfrog tadpoles.

Composition	Corn	Peeled banana	Peeled avocado	Peeled pumpkin
Moisture (g/kg)	120.5	186.9	55.5	109.2
Protein (g/kg)	74.0	34.4	54.9	130.1
Fat (g/kg)	32.8	4.4	493.5	47.7
Ash (g/kg)	9.1	25.0	45.1	56.1
Fiber (g/kg)	55.0	9.7	59.6	63.7
Starch (g/kg)	708.6	739.6	291.4	593.2
Gross energy (kcal/kg)	3,954.07	3,714.02	7,177.04	3,769.50

Table 2. Bullfrog tadpole diets containing peeled banana, pumpkin, and avocado meals as substitutes for 10% corn.

Ingredient	Corn	Banana	Avocado	Pumpkin
Salmon meal (g/kg)	178.8	178.8	178.8	178.8
Soybean meal (g/kg)	250.0	250.0	250.0	250.0
Corn gluten (g/kg)	200.0	200.0	200.0	200.0
Corn (g/kg)	130.0	30.0	30.0	30.0
Wheat bran (g/kg)	105.0	105.0	105.0	105.0
Corn starch (g/kg)	100.0	100.0	100.0	100.0
Peeled banana (g/kg)	-	100.0	-	-
Peeled pumpkin (g/kg)	-	-	-	100.0
Peeled avocado (g/kg)	-	-	100.0	-
Soybean oil (g/kg)	30.0	30.0	10.0	30.0
Inert (sand) (g/kg)	-	-	20.0	-
Premix[1]	6.0	6.0	6.0	6.0
BHT	0.2	0.2	0.2	0.2
Total (kg)	1,000.0	1,000.0	1,000.0	1,000.0
Crude protein (g/kg)	342.6	338.7	340.7	348.3
Digestible protein (g/kg)	304.2	300.1	301.7	307.8
Gross energy (kcal/kg)	4,478.58	4,454.58	4,618.36	4,460.12
Digestible energy (kcal/kg)	3,561.87	3,507.27	3,665.08	3,511.71
Ether extract (g/kg)	61.3	58.5	87.4	62.8

*Values calculated based on the digestibility coefficients reported by Secco et al. (2005).

[1]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 (µg/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 (%) 71.64; magnesium (%) 0.0085; sulfur (%) 1.159; 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.

Measurements

Biometric measurements were taken at the beginning and at 15, 30, 45, and 60 days of the experiment and consisted of the measurement of individual tadpole weight (g) on a digital scale (0.001 g) and total and standard length using a digital caliper (0.01 mm). Additionally, metamorphic development was evaluated based on Gosner stages (1960) with a trinocular stereoscopic microscope equipped with 10× to 40× lenses.

The following performance parameters were evaluated: body weight, standard and total lengths, and metamorphic development obtained during each measurement (1, 15, 30, 45, and 60 days); and weight gain, feed intake, feed conversion, specific growth rate, and survival rate for the entire experimental period (1–60 days).

Water quality was monitored by measuring water temperature, total ammonia (NH_3 + NH_4), toxic ammonia (NH_3), and pH during the periods described above (Table 3).

At the end of the experiment, 30 g of tadpole live weight from each replication was sampled for analysis of percentage composition (water, protein, ash, and fat).

Table 3. Water quality in the rearing tanks of bullfrog tadpoles fed diets containing peeled banana, pumpkin, and avocado meals as substitutes for corn.

Ingredient	Water temperature (°C)	NH_3+NH_4 (mg/L)	NH_3 (mg/L)	pH
Corn	24.3±0.1	0.204±0.012	0.0016±0.0001	7.19±0.02
Banana	24.4±0.1	0.195±0.006	0.0015±0.0001	7.20±0.01
Avocado	24.4±0.1	0.191±0.012	0.0016±0.0001	7.18±0.01
Pumpkin	24.5±0.1	0.026±0.010	0.0016±0.0001	7.18±0.01
P-value	*0.5679*	*0.7254*	*0.9126*	*0.8025*

Sample processing and laboratory tests

After 60 days of the experiment, tadpole samples (whole body) were ground in a meat processor and stored in labeled jars in a freezer at -10 °C. Dry matter was determined in an oven at 105 °C (Silva and Queiroz, 2006). Crude protein was analyzed using the Kjeldahl method (AOAC, 1984); crude fat using the Folch method (Folch et al. 1957); and ash by incineration in a muffle furnace at 600 °C (Silva and Queiroz, 2006).

Statistical analysis

Performance and percentage composition data were analyzed using the Shapiro–Wilk and Bartlett tests to check normality and homoscedasticity of the data. Results were subjected to analysis of variance, and the mean values were compared using the Duncan test at the 5% level of significance, except for metamorphic development, in which the stages of metamorphosis underwent linear regression as a function of age or time of the experiment (days). All statistical procedures were performed using SAS 9.2. software (SAS, 2008).

RESULTS

Differences in body weight between diets were observed at day 30 of the

experiment ($p < 0.05$), particularly in animals fed banana meal diet (1.85 ± 0.06 g). After 45 days, tadpoles fed pumpkin meal diet had a similar body weight (1.93 ± 0.04 g) to that of animals fed banana meal diet, a pattern that continued until the end of the experiment. The body weight of tadpoles receiving the control diet (1.63 ± 0.08 g), which had a higher corn content, was similar to that of animals fed avocado meal diet (1.49 ± 0.04 g) throughout the experimental period (Table 4).

Table 4. Weight, total length, and standard length of bullfrog tadpoles fed diets containing peeled banana, pumpkin, and avocado meals as substitutes for corn.

Diet	1 day	15 days	30 days	45 days	60 days
	Body weight (g)				
Corn	0.12±0.01	0.33±0.01	0.57±0.02 b	1.02±0.07 b	1.63±0.08 b
Banana	0.10±0.01	0.32±0.04	0.69±0.02 a	1.25±0.03 a	1.85±0.06 a
Avocado	0.10±0.01	0.28±0.01	0.57±0.01 b	0.95±0.04 b	1.49±0.04 b
Pumpkin	0.10±0.01	0.33±0.03	0.62±0.04 b	1.29±0.07 a	1.93±0.04 a
p value	*0.2054*	*0.3788*	*0.0117*	*0.0016*	*0.0004*
	Total length (mm)				
Corn	19.03±0.50	34.04±0.72	40.65±1.67	50.31±1.01	59.17±2.43 b
Banana	19.57±0.24	31.03±2.63	43.12±1.83	53.07±0.69	61.41±2.13 b
Avocado	19.61±1.68	32.55±1.96	41.44±0.76	49.61±1.28	56.84±1.64 b
Pumpkin	21.05±1.04	32.99±1.59	44.19±0.87	54.39±1.89	66.88±1.14 a
p value	*0.3942*	*0.7202*	*0.2968*	*0.0699*	*0.0164*
	Standard length (mm)				
Corn	8.72±0.27	13.81±0.39	16.76±0.53	20.57±0.23	23.89±0.82 b
Banana	8.64±0.32	13.19±0.84	17.70±0.84	21.29±0.50	25.22±0.25 a
Avocado	8.62±0.28	13.35±0.73	16.78±0.24	19.68±0.50	22.23±0.35 b
Pumpkin	9.43±0.47	13.01±0.46	17.55±0.31	21.76±0.89	26.54±0.29 a
p value	*0.3257*	*0.8261*	*0.4793*	*0.1122*	*0.0003*

[1]Mean values in the same column followed by different lowercase letters differ using Duncan's test ($p < 0.05$).

A significant difference in the total length of bullfrog tadpoles (Table 4) was only observed at the end of the experiment, when tadpoles fed pumpkin meal diet (66.88 ± 1.64 mm) displayed a better result ($p < 0.05$) than the control (59.19 ± 2.43 mm) and animals receiving the banana (61.41 ± 2.13 mm) and avocado meal diets (56.84 ± 1.64 mm). Differences in mean standard length were also observed only at the end of the experiment. However, the standard length was similar in tadpoles fed banana (25.22 ± 0.25 mm) and pumpkin meal diets (26.54 ± 0.29 mm); both groups showed higher values than those fed the control (23.89 ± 0.82 mm) and avocado meal diets (22.23 ± 0.25 mm) (Table 4).

Weight gain, feed intake, feed conversion, and specific growth rate at 60 days were higher ($p < 0.05$) in tadpoles fed pumpkin and banana meal diets than those in the control and tadpoles fed avocado meal diets. Feed conversion was similar ($p > 0.05$) for all treatments, since tadpoles that gained more weight consumed more feed and

those that consumed less feed grew less. An excellent survival rate above 90% was observed for all diets, with no significant differences ($p > 0.05$) between treatments (Table 5).

Table 5. Weight gain, feed intake, feed conversion, specific growth rate, and survival of bullfrog tadpoles fed diets containing peeled banana, pumpkin, and avocado meal as substitutes for corn.

Ingredient	Weight gain (g)	Feed intake (g)	Feed conversion (g/g)	Specific growth rate (%/day)	Survival rate (%)
Corn	1.51±0.08 b	2.15±0.07 b	1.44±0.04	4.64±0.13 b	91.7±0.96
Banana	1.75±0.06 a	2.44±0.08 a	1.40±0.08	5.20±0.16 a	93.3±2.36
Avocado	1.39±0.03 b	1.99±0.06 b	1.43±0.05	4.79±0.11 b	95.0±0.96
Pumpkin	1.83±0.04 a	2.43±0.13 a	1.33±0.10	5.22±0.15 a	94.0±2.10
p value	*0.0004*	*0.0112*	*0.7213*	*0.0222*	*0.2230*

[1]Mean values in the same column followed by different lowercase letters were different using Duncan's test ($p < 0.05$).

The body composition of bullfrog tadpoles differed between treatments ($p < 0.05$). Tadpoles fed avocado meal diet showed a higher moisture content than the other treatments and control. Protein deposition was lower in tadpoles fed pumpkin meal diet than that in the other groups. Tadpoles fed avocado meal diet showed an ether extract content similar to that observed in animals fed pumpkin meal diet ($p > 0.05$) and lower than that found in animals fed only the corn and banana meal diets ($p < 0.05$). Taken together, the body composition results reveal that these alternative feeds induce a different metabolic behavior in bullfrog tadpoles (Table 6).

Table 6. Moisture, protein, ether extract, and ash content of bullfrog tadpoles fed diets containing peeled banana, pumpkin, and avocado meals as substitutes for corn.

Diet	Moisture (%)	Protein (%)	Ether extract (%)	Ash (%)
Corn	87.29±0.26 b	7.35±0.45	3.37±0.13 a	1.42±0.03
Banana	87.55±0.35 b	6.53±0.32	3.24±0.13 a	1.36±0.05
Avocado	88.83±0.33 a	6.59±0.22	2.69±0.12 c	1.39±0.06
Pumpkin	87.87±0.23 b	6.14±0.67	2.99±0.10 b	1.37±0.03
p value	*0.0035*	*0.0601*	*0.0116*	*0.8143*

[1]Mean values in the same column followed by different lowercase letters differ using Duncan's test ($p < 0.05$).

Linear regression analysis of tadpole metamorphic stages revealed normal development for all treatments and controls (Figure 1). Comparative evaluation between treatments was not performed; however, the tadpoles that received the novel feeds were in more advanced stages than the control, especially those fed banana and pumpkin flour diets.

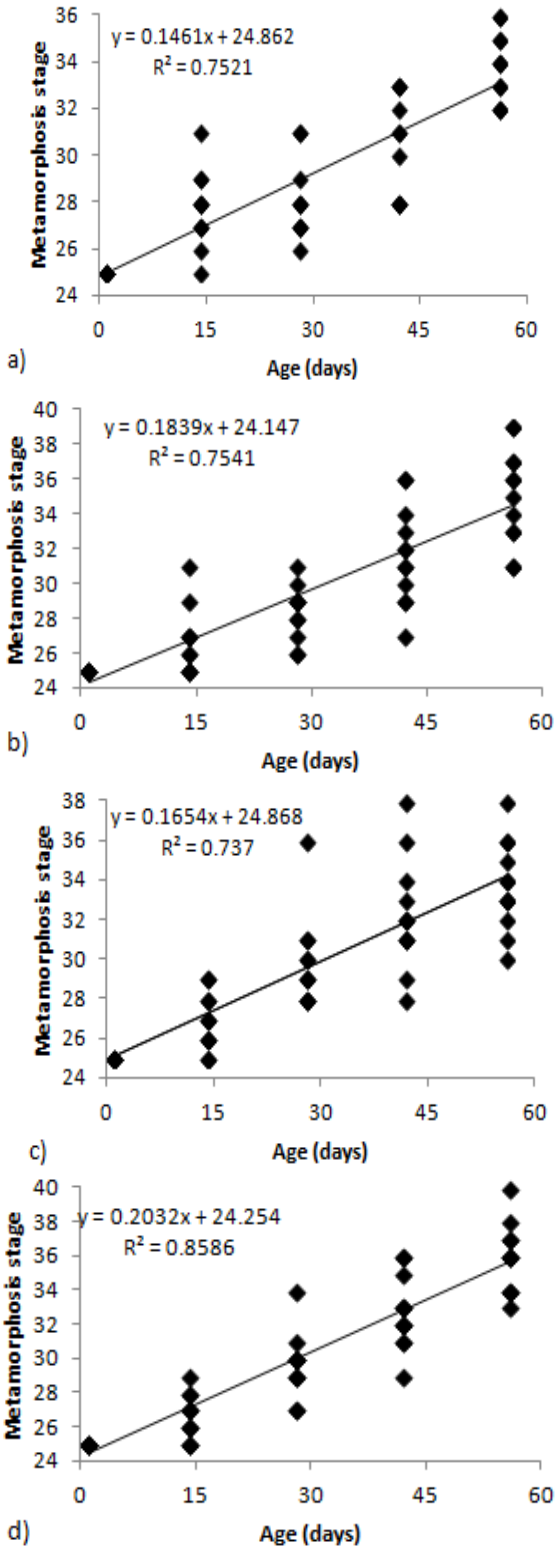

Figure 1. Developmental stages (Gosner, 1960) of bullfrog tadpoles fed diets containing (a) corn, (b) peeled banana meal, (c) avocado meal, and (d) and pumpkin meal as substitutes for corn.

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DISCUSSION

Analysis of body weight; total and standard lengths; weight gain; feed intake; specific growth rate; survival rate; moisture, protein, ether extract, and ash contents; water quality; and metamorphosis stages of bullfrog tadpoles showed better results for animals receiving peeled banana and pumpkin meals as substitutes for corn. The superiority of some diets can be explained by the nutrient composition of the corn substitutes (Table 1).

The inferior performance of animals on avocado meal compared with the other meals might be related to its high lipid content (493.5 g/kg). Tadpoles are animals that undergo constant anatomical and physiological changes. At this stage of life, the digestive system is still not prepared with sufficient lipolytic enzymes to break down this high content of lipids and allow their absorption (Seixas Filho et al., 2008c, 2010). However, avocado meal displayed a similar result to that of corn and therefore met the objective of this study, i.e., to identify substitutes for ground corn in diets for bullfrog tadpoles.

Furthermore, studies of enzymatic activities in bullfrog tadpoles receiving commercial diets with 28%, 32%, 36%, and 40% crude protein have demonstrated a higher specific activity of amylase at 18, 26, 35, and 55 days of the experiment than that with lipase, which showed higher activity than trypsin (Seixas Filho et al., 2011). This knowledge confirms the potential of feed ingredients like peeled banana and peeled pumpkin for tadpole feeding as they contain a high amount of starch.

Peeled banana meal has a high starch content (739.6 g/kg) and low crude protein (34.4 g/kg) and fiber (9.7 g/kg) contents. Peeled pumpkin meal also has high starch (593.2 g/kg), medium crude protein (130.0 g/kg), and high fiber (63.7 g/kg) content. Corn, the replaced ingredient, is characterized by high levels of starch (708.6 g/kg), low crude protein (74.0 g/kg), and high fiber (63.7 g/kg). The three novel feed ingredients are high in starch, but peeled banana meal has a low fiber content, and peeled pumpkin meal, despite its high fiber content, has a medium crude protein content, which may compensate for the high levels of fiber. This information may explain the better performance of omnivorous bullfrog tadpoles fed these meals. An investigation of different plant sources (broken rice, ground corn, wheat bran, citrus pulp, and soybean hulls) containing different levels of dietary fiber in the omnivorous fish species, tilapia and catfish, showed a reduction in protein, energy, and dry matter digestibility with increasing fiber content in both species (Rodrigues et al., 2012).

Another difference between the peeled banana, avocado, and pumpkin meals and corn is their macro- and micronutrient levels. The total ash content of corn is 9.1 g/kg, while this value is 25.0 g/kg for banana meal, 45.1 g/kg for avocado meal, and 56.1 g/kg for pumpkin meal, with the potassium content of pumpkin meal corresponding to 75% of total ash. However, it should be noted that the corn used in this study was an excellent source of magnesium and calcium. The future adoption of these meals by the feed industry would be an interesting strategy to offset the

mineral deficiency of some feed ingredients.

The water (87.29% to 88.83%), protein (6.14% to 7.35%), fat (2.69% to 3.37%), and ash (1.36% to 1.42%) content of tadpoles fed corn and peeled banana, avocado, and pumpkin meal correspond to studies that determined the protein requirement for bullfrog tadpoles (Pinto et al., 2015). Additionally, they correspond to studies that compared the nutrient content between a commercial and an experimental diet for bullfrog tadpoles (Mansano et al., 2014), and research evaluating the nutrient deposition using a commercial fish diet containing 40% crude protein (Mansano et al., 2013).

Histological studies of the organs of bullfrog tadpoles have shown that diets formulated for fish can cause organ damage in the tadpoles, even those containing low protein (Seixas Filho et al., 2008a). Proteins of low biological value compromise animal health and performance (Seixas Filho et al., 2008b). The performance of the tadpoles fed peeled avocado, pumpkin, and banana meal as substitutes for corn may indicate improvement in the quality of the diets, but histological studies are necessary.

A decline in water temperature was observed throughout the experiment for all treatments, especially in the last fortnight (22.7 °C). This had an effect on performance, particularly on specific growth rate (Table 4), since the ideal water temperature for raising bullfrog tadpoles is 25 °C (Hoffmann et al., 1989). However, the overall mean temperature (24.4 °C) was close to the thermal comfort zone (Table 6). The remaining parameters related to water quality, such as toxic ammonia (NH_3) levels, total ammonia (NH_3+NH_4), and pH, displayed acceptable values (Table 6) for the maintenance of homeostasis in bullfrog tadpoles (Seixas Filho et al., 2012; Borges et al., 2014).

CONCLUSION

The performance of bullfrog tadpoles fed peeled banana, avocado, and pumpkin meals made viable the inclusion of these meals as substitutes for corn in tadpole diets.

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