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# Optimal digestible protein level for bullfrog tadpoles

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ABSTRACT

In view of the importance of tadpole rearing to obtain good results of frog farming, the objective of this study was to determine the optimal digestible protein (DP) level for bullfrog tadpoles. Tadpoles were fed isoenergetic diets (15.4 kJ DE $g^{-1}$) containing 21, 23, 25, 27, 29 and 31% DP. The following parameters were evaluated: weight gain, feed intake, dietary protein intake, feed conversion, specific growth rate, protein efficiency ratio, and deposition of protein, fat, water and ashes. The optimal level of DP for weight gain estimated by broken-line regression analysis was 27.7%. Feed intake decreased and protein intake, specific growth rate and feed conversion increased with increasing levels of DP in the diet. No significant difference was observed for protein efficiency ratio. Tadpoles fed diets containing 27, 29 and 31% DP exhibited higher deposition of protein, water and ashes and lower fat deposition. The results suggest 27.7% DP for bullfrog tadpoles.



## 1. Introduction

Frog farming has an economic growth potential for the domestic and international market (FAO, 2010), which is boosted by the increase in the consumption of white and healthy meat as an alternative source of protein (Moreira et al., 2013).

Frog production is divided into the following phases: breeding, tadpole rearing, and frog fattening. In this respect, tadpole production is an important step since it is an intermediate phase of the bullfrog life cycle (Mansano et al., 2012) and precedes a critical period, i.e., metamorphosis (Scott et al., 2007). Furthermore, after metamorphosis, the froglet weighs less than the tadpole at the climax of metamorphosis (Stamper et al., 2009), and the heavier the froglet, the better will be its fattening performance (Álvarez and Real, 2004).

Tadpoles require adequate feeding to gain weight; however, the nutritional requirements of tadpoles are not completely established. As a consequence, tadpoles are fed commercial rations used for carnivorous or omnivorous fish (Castagna et al., 2014; Seixas Filho et al., 2010, 2011, 2013). These rations can cause abnormal development, death, classical signs of nutritional disorders, and histopathological alterations in organs such as the liver, intestine, stomach and kidneys (Seixas Filho et al., 2008). Mansano et al. (2013) observed that the protein of a commercial diet (57.53% crude protein) was not fully utilized by bullfrog tadpoles and that the intake of nutrients can be 10 times higher than their deposition in the carcass.

Studies have investigated the nutrition of bullfrog tadpoles. Carmona-Osalde et al. (1996) determined a crude protein requirement of 44.61% in an assay testing six levels of crude protein (30, 35, 40, 45, 50 and 55%) and adopting a practical diet. This value can be considered high since bullfrog tadpoles are omnivorous animals (Pryor and Bjorndal, 2005). Seixas Filho et al. (1998) observed the same performance and survival in bullfrog tadpoles fed diets with different energy levels (4200 kcal CE $kg^{-1}$ and 3300 kcal ME $kg^{-1}$) and crude protein (25, 35, and 45%); however, the authors used metabolizable energy of carp (*Cyprinus carpio*) to formulate the diets. In another study, Seixas Filho et al. (2010) obtained a better weight for tadpoles fed a commercial diet containing 45% crude protein. However, the quality of the protein was not considered. In this respect, the digestibility of protein and energy foods for bullfrog tadpoles has also been investigated (Albinati et al., 2000; Secco et al., 2005). Seixas Filho et al. (2012), testing three levels of digestible protein (27, 31, and 35%), only observed a higher protein efficiency ratio for tadpoles fed the diet containing 27% digestible protein.

In view of the high cost of dietary protein sources and the need for a specific diet, the objective of the present study was to determine the optimal digestible protein level for bullfrog tadpoles.

## 2. Materials and methods

### 2.1. Experimental conditions and animals

The experiment was conducted at the Aquiculture Center of the Paulista State University (Universidade Estadual Paulista — UNESP),

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Jaboticabal Campus, over a period of 80 days, between November 2012 and January 2013.

A total of 9120 bullfrog tadpoles (*Lithobates catesbeianus*) in Gosner stage 25 (1960), initially weighing 0.015 ± 0.02 g, were housed in 24 experimental boxes (190 l, 2 tadpoles/l) equipped with a closed recirculation system and renewal of 100% of its volume at intervals of 24 h. The water was obtained from an artesian well.

The water quality in the boxes was maintained by siphoning off feces and unconsumed ration on alternate days. The maximum and minimum temperatures were measured daily with a digital maximum and minimum thermometer. Dissolved oxygen (YSI professional oxygen meter), electrical conductivity (PHTEK CD-203 portable conductivity meter), and pH (PHTEK pH-100 portable pH meter) were measured weekly.

### 2.2. Study design

The tadpoles were distributed in a completely randomized design consisting of six treatments, corresponding to the levels of digestible protein (21, 23, 25, 27, 29, and 31%), and four replicates.

### 2.3. Experimental diets

The tadpoles received isoenergetic diets (15.4 kJ DE $g^{-1}$) containing 21, 23, 25, 27, 29, and 31% digestible protein (Table 1). The digestibility coefficients for protein and energy of the ingredients were obtained by Secco et al. (2005).

For preparation of the diets, the ingredients were ground in a grinder using a sieve with a pore size of 1.7 mm. The diets were then pelleted in a pellet mill (California Pellet Mill Co.) and the pellets were ground again (1.7 mm).

The experimental diets were offered six times per day (8:00, 10:00, 12:00, 14:00, 16:00, and 18:00 h) until apparent satiety of the tadpoles. Leftovers were avoided so that the amount offered was considered to be equivalent to the amount consumed (Solomon and Taruwa, 2011). The daily amount offered was recorded for the calculation of feed intake.

**Table 1**
Composition of the diets used for bullfrog tadpoles.

| | Diet (% digestible protein) | | | | | |
|---|---|---|---|---|---|---|
| | 21 | 23 | 25 | 27 | 29 | 31 |
| *Ingredients (%)* | | | | | | |
| Fish meal | 12.1 | 13.2 | 14.4 | 15.5 | 16.7 | 17.8 |
| Poultry by-product meal | 9.7 | 10.6 | 11.5 | 12.5 | 13.4 | 14.3 |
| Soybean meal | 8.3 | 11.8 | 13.6 | 17.3 | 20.0 | 21.0 |
| Corn | 40.0 | 35.8 | 35.0 | 31.0 | 27.9 | 26.0 |
| Wheat meal | 16.0 | 15.0 | 16.5 | 15.0 | 15.5 | 19.7 |
| Starch | 8.1 | 9.2 | 5.3 | 6.6 | 5.4 | 0.2 |
| Soybean oil | 5.3 | 3.8 | 3.2 | 1.6 | 0.6 | 0.5 |
| Mineral and vitamin supplement[a] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Bht | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Digestible protein [b] (%) | 21.0 | 23.0 | 25.0 | 27.0 | 29.0 | 31.0 |
| Digestible energy [b] (kJ $g^{-1}$) | 15.4 | 15.4 | 15.4 | 15.4 | 15.4 | 15.4 |
| *Composition analyzed* | | | | | | |
| Crude protein (%) | 24.1 | 26.1 | 26.7 | 30.3 | 33.0 | 33.8 |
| Crude energy (kJ $g^{-1}$) | 17.9 | 18.3 | 17.8 | 17.9 | 17.8 | 17.9 |
| Mineral matter (%) | 5.5 | 4.4 | 4.4 | 7.8 | 7.7 | 8.6 |
| Ether extract (%) | 11.8 | 12.6 | 10.3 | 9.0 | 8.3 | 8.4 |

[a] Moisture (%) 2.0; ash (%) 71.6442; vitamin A (min) 35,000 IU; vitamin D3 (min) 2000 IU; vitamin E (min) 120 IU; vitamin K3 (min) 800 mg; folic acid (min) 10 mg; biotin (min) 10 mg; thiamine (B1) (min) 25 mg; riboflavin (B2) (min) 35 mg; pyridoxine (B6) (min) 40 mg; vitamin B12 (min) 100 μg; niacin (min) 350 mg; pantothenic acid (min) 150 mg; choline (min) 2500 mg; copper (min) 25 mg; iron (min) 150 mg; manganese (min) 75 mg; selenium (min) 1 mg; zinc (min) 140 mg; mannan-oligosaccharide (min) 60 mg.

[b] Values calculated from the digestibility coefficients of Secco et al. (2005).

### 2.4. Parameters analyzed

Biometry was performed at the beginning and at the end of the experiment. At the beginning of the experiment, 10% of the tadpoles and at the end all tadpoles of each experimental box were weighed individually on a digital electronic scale (precision of 1 mg).

The initial and final biometry data and the feed intake data per experimental box were used to evaluate feed intake, dietary protein intake, feed conversion, specific growth rate, and protein efficiency ratio. The mortality rate of the tadpoles was also evaluated.

For the determination of initial body composition (protein, ether extract, ashes, and dry matter), a representative sample of 100 tadpoles of the same group of animals were sacrificed. At the end of the experiment, 10% of the tadpoles of each experimental box were placed in containers with water for 24 h for elimination of gastrointestinal tract content. Next, the tadpoles were stunned on ice, euthanized, and frozen for subsequent analysis. The body composition data were used to calculate the deposition of protein and fat (Mansano et al., 2014).

### 2.5. Sample processing and laboratory analysis

The tadpole samples were ground in a food processor, stored on Petri dishes, and lyophilized at −50 °C to obtain dry matter. Next, the samples were ground in a ball mill and sent to the laboratory for analysis of crude protein by the Dumas method using a Leco 528 LC apparatus (Etheridge et al., 1998). Ether extract was determined by extraction with petroleum ether in a Soxhlet apparatus. Ashes were measured in a muffle at 550 °C by incineration (Silva and Queiroz, 2002).

All procedures were approved by the Ethics Committee on Animal Use of the School of Agricultural and Veterinary Sciences, UNESP (Permit No. 010.478/13), and were conducted in accordance with the ethical guidelines of the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal — COBEA).

### 2.6. Statistical analysis

The body composition data (water, protein, fat, and ashes) and mortality rate of the bullfrog tadpoles were analyzed regarding normality and homoscedasticity by the Shapiro–Wilk and Bartlett tests, respectively. The data were submitted to analysis of variance (ANOVA) and, when significant ($p < 0.05$), to the Duncan test ($p = 0.05$). The PROC ANOVA procedure of the SAS 9.2 software (SAS Institute, 2008) was used for analysis.

The results of the other parameters were submitted to regression analysis to determine the best values. A linear response plateau (LRP) model or broken-line regression analysis was adopted: $Y = L + U (R - X)$, where $Y$ = value of the variable studied; $X$ = level of digestible protein in the diet; $L$ = plateau response of the variable studied; $U$ = slope of the line; and $R$ = level of digestible protein estimated by the breakpoint (Robbins et al., 1979). Linear regression was performed when no convergence of the data was obtained with the equation described above: $Y = A X + B$, where $Y$ = value of the variable studied; $A$ = linear regression coefficient; $X$ = level of digestible protein in the diet; and $B$ = constant corresponding to the intercept of the line with the vertical axis. The SAS 9.2 program was used for statistical analysis, including the PROC REG procedure to fit the linear regressions and the PROC NLIN procedure to fit the LRP or broken-line regression (SAS Institute, 2008).

Weight gain percentage (WGP) and protein deposition were used to determine the optimum level of digestible protein for bullfrog tadpoles. The remaining parameters were used to observe the response to the different diets.