NUTRIENT DEPOSITION IN BULLFROGS DURING THE FATTENING PHASE*

Marcelo Maia PEREIRA[1]; Cleber Fernando Menegasso MANSANO[1]; Nelson José PERUZZI[2]; Marta Verardino De STÉFANI[1]

ABSTRACT

Descriptions of nutrient deposition are important to determine the nutritional requirements of animals. The objective of this study was to describe nutrient deposition in the body and legs and the dietary intake of protein and fat of bullfrogs during the fattening phase using Gompertz and logistic models, and to evaluate feed efficiency. A total of 2,375 froglets with an initial weight of 7.03 ± 0.16 g were housed in five fattening pens. The animals were fed a commercial diet containing 40% crude protein. Frogs and their legs were weighed and sampled at intervals of 14 days for the determination of nutrient composition. On the basis of the model selection criteria, the logistic model was the most adequate to describe nutrient deposition in the body and legs of bullfrogs and the dietary intake of protein and fat. With respect to nutrient deposition in the body, the estimated values for nutrient weight at maturity (Wm) and the time when the maximum rate of deposition was attained (t*) were 244.3 g and 106 days, 55.2 g and 113 days, 30.9 g and 124 days, and 8.6 g and 99 days for water, protein, fat and ashes, respectively. For nutrient deposition in the legs, these values were 77.6 g and 111 days, 14.5 g and 104 days, 1.4 g and 86 days, and 3.7 g and 119 days, respectively. The protein efficiency of the bullfrog diet was low (36.76%), whereas the efficiency of fat utilization was high (140.9%), indicating the need to develop an ideal diet for bullfrogs.

Keywords: *Lithobates catesbeianus*; fat deposition; logistic; protein deposition

DEPOSIÇÃO DE NUTRIENTES EM RÃ-TOURO NA FASE DE ENGORDA

RESUMO

As descrições da deposição dos nutrientes são importantes para auxiliar na determinação das exigências nutricionais para os animais. A partir do exposto, o objetivo do presente estudo foi descrever a deposição dos nutrientes no corpo e nas pernas da rã-touro, bem como o consumo proteico e de gordura da dieta, através dos modelos de Gompertz e Logístico, e avaliar a eficiência da dieta para rã-touro na fase da engorda. Foram utilizados 2.375 imagos de rã-touro com peso inicial de 7,03 ± 0,16 g, alojados em cinco baias de engorda. Os animais foram alimentados com dieta comercial com 40% de proteína bruta. A cada 14 dias, foram realizadas pesagens e amostragens das rãs e de suas pernas, para obtenção da composição dos nutrientes. A partir dos critérios de avaliação, o modelo Logístico foi o mais adequado para descrever a deposição de nutrientes no corpo e pernas das rãs, bem como o consumo de proteína e gordura da dieta. Os valores estimados de peso dos nutrientes à maturidade (Pm) e o tempo onde a taxa de deposição foi máxima (t*) para água, proteína, gordura e cinzas do corpo da rã-touro foram: 244,3 g e 106 dias; 55,2 g e 113 dias; 30,9 g e 124 dias; 8,6 g e 99 dias, respectivamente. Para as pernas, os valores foram: 77,6 g e 111 dias; 14,5 g e 104 dias; 1,4 g e 86 dias; 3,7 g e 119 dias, respectivamente. A dieta comercial apresentou uma baixa eficiência proteica (36,76%) e alta eficiência da utilização da gordura (140,9%) para rã-touro, sendo necessário o desenvolvimento de uma dieta ideal para a mesma.

Palavras chave: *Lithobates catesbeianus*; deposição de gordura; logístico; deposição de proteína

---

Artigo Científico: Recebido em 20/05/2014 – Aprovado em 14/05/2015

[1] *Aquaculture Center (CAUNESP), São Paulo State University (UNESP). Via de Acesso Prof. Paulo Donato Castellane, s/n – CEP: 14.884-000 – Jaboticabal – SP – Brazil. e-mail: mmaiap2001@yahoo.com.br (corresponding author); clebermansano@yahoo.com.br; martavs@fcav.unesp.br*

[2] *Department of Animal Science, College of Agrarian and Veterinary Sciences, São Paulo State University (UNESP). Jaboticabal – SP – Brazil. e-mail: peruzzi@fcav.unesp.br*

* *Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Grant 11/50865-2); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (doctoral fellowship to the first author).*

INTRODUCTION

The meat of bullfrogs, the main species reared on frog farms in producer countries around the world, is characterized by high protein and low fat content (TOKUR *et al*., 2008). The legs are the edible part and the international trade of frog legs has an estimated value of 40 million dollars per year (TURNIPSEED *et al*., 2012). The main consumers are France and the United States (NEVEU, 2009).

The life cycle of the bullfrog consists of two very distinct phases, an aquatic and a terrestrial phase. The latter is characterized by carnivorous eating habits and high protein requirements for growth (OLVERA-NOVOA *et al*., 2007). Since the nutritional requirements of the bullfrog are not well defined, rations for carnivorous fish are used on frog farms which are not specific for frogs (CASALI *et al*., 2005; FENERICK JÚNIOR and STÉFANI, 2005).

The growth of production animals over time in response to a specific treatment or to the environment where the animal lives can be described using mathematical equations whose parameters can be interpreted in biological terms. For example, in bullfrogs, Gompertz and logistic models have shown the best fit to describe the live weight gain of animals (RODRIGUES *et al*., 2007). In addition to live weight, nonlinear models can also be fit to body components (protein, fat, water, and ashes), permitting the construction of curves for the deposition of each nutrient (MARCATO *et al*., 2008; CASAS *et al*., 2010). In this respect, a mass increase in body components or nutrients determines the growth of animals.

Knowledge of the deposition of body nutrients in the animal provides a better understanding in nutrient partitioning and important information for researchers in the field of nutrition. Growth models and the understanding of the processes of nutrient deposition in fish have contributed to the formulation of fish diets (HUA *et al*., 2010). The growth and efficiency with which nutrients are deposited and utilized are affected by endogenous (species, genetic factors, and stage of life) and exogenous factors (diet composition, rearing environment, among others) (DUMAS *et al*., 2010).

In view of the above considerations, the objective of the present study was to describe the curves of nutrient deposition in the body and legs and the dietary intake of protein and fat of bullfrogs during the fattening phase using Gompertz and logistic models. In addition, the efficiency of protein and fat intake was evaluated.

MATERIAL AND METHODS

Study place

The experiment was conducted at the Aquaculture Center of the Paulista State University (Universidade Estadual Paulista – UNESP), Sector of Raniculture, between October 2010 and February 2011. The analyses of body nutrients were performed in the Laboratory of Animal Nutrition (Laboratório de Nutrição Animal - LANA), Department of Zootechny, School of Agricultural and Veterinary Sciences (Faculdade de Ciências de Agrárias e Veterinárias – FCAV), UNESP.

The bullfrogs were housed in five fattening pens (12 m^2) containing shelters, a small water trough and vibrating feeders in a linear arrangement (LIMA, 1997). Continuous water flow was provided from an artesian well.

Animals and farming methods

A total of 2,375 bullfrog froglets (*Lithobates catesbeianus*) with a live weight of 7.03 ± 0.16 g were used. The density in each fattening pens initially was 39 bullfrogs m^{-2} and the final was around 21 bullfrogs m^{-2}.

The frogs were fed daily a commercial extruded diet developed for tropical freshwater fish (Manufacturer information: centesimal composition = 39.65% crude protein; 4,366.3 kcal. kg^{-1} crude energy; 4.89% ether extract; 1.91% crude fiber; 10.28% mineral matter and 6.13% water. Basic diet composition: soybean meal, wheat bran, corn gluten meal 60, fish meal, ground whole corn, vegetable fat, stabilizer, limestone, dicalcium phosphate, Refinazil, and mineral-vitamin premix. Possible substitutes: rice bran, maize germ, rice grits, ground whole grain sorghum, sugar cane dry yeast, meat and bone meal, hydrolyzed feather meal, visceral meal, and blood meal. Enrichment per kg product: vitamin A, 16,000 IU kg^{-1}; vitamin D, 4,500 IU kg^{-1}; vitamin E, 250 mg; vitamin K, 30 mg; vitamin C, 350 mg; thiamine (B1), 32 mg; riboflavine (B2), 32 mg; pyridoxine (B6), 32 mg; vitamin B12, 32 mg; niacin, 170 mg, biotin 10 mg, folic acid, 10 mg; calcium pantothenate, 80 mg; choline, 2,000 mg; cobalt, 0.5 mg; copper, 20 mg; iron, 150 mg; iodine, 1 mg; manganese, 50 mg; selenium 0,7 mg, and zinc, 150 mg).

Leftovers from the feeders were removed, dried in an oven at 55 °C for 24 hours, and weighed for the calculation of food intake by the animals. The size of the pelleted ration ranged from 2 to 4 mm during the first 45 days and from 6 to 8 mm thereafter. The feeders were cleaned daily. The water trough of the pens was also emptied and cleaned daily, and the water was renewed (100%). 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.

The water and ambient temperature was measured daily with a minimum/maximum thermometer placed 30 cm from the floor, with the sensor being attached to the apparatus inside the water.

Biometry and sampling

Ten samplings were performed at intervals of 14 days. In the first sampling, 40 froglets were sampled from the initial batch, followed by 40 animals/pen in the second and third sampling and 20 animals/pen in the fourth to tenth sampling. The sampled animals were stunned on ice, weighed on a digital scale (0.01 g), and the spine was sectioned.

In half the frogs sampled, the skin was removed and the legs were cut (muscles and bones), weighed and frozen for subsequent analysis. In the other half, the entire frog was frozen for 24 hours. After this period, the celomatic cavity was opened and the digestive tract was removed, emptied and returned to the carcass, which was again frozen for subsequent analysis.

All procedures were approved by the Ethics Committee on Animal Use of FCAV-UNESP (Protocol Nº 024999/10) and were conducted in accordance with the ethical guidelines of the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal - COBEA).

Sample processing and laboratory analysis

The frozen animals and legs were ground in an industrial meat grinder to obtain homogenous samples. A subsample (100 g) was removed, transferred to a disposable plastic Petri dish, and lyophilized at -50 ºC in a Thermo VLP200 lyophilizer to obtain dry matter. Next, the sample was again ground in an IKA microfine grinding mill and sent to the laboratory for analysis of nitrogen, ether extract, dry matter, ashes, and crude energy (only carcass).

Crude protein in the samples was determined by the method of Dumas in a Leco 528 LC apparatus (ETHERIDGE *et al*., 1998). For ether extract analysis, extraction was performed with petroleum ether in a Soxhlet apparatus. Ash content was determined by incineration in a muffle furnace at 550 ºC, and dry matter content was obtained by incubation in an oven at 105 ºC for 12 hours. Crude energy was determined with a Parr calorimeter. The methods used were described by SILVA and QUEIROZ (2006).

Statistical analysis

A completely randomized design consisting of five experimental units were used, with the repetitions being.

The contents of crude energy, moisture or water, protein, ether extract and ashes in the carcass and legs were analyzed by polynomial regression, where the Y-axis = crude energy (kcal kg^{-1}) or nutrients (%) in the frog or legs, and X-axis = time or age in days. The PROC REG procedure (p = 0.05) of the Statistical Analysis System software (SAS, 2008) was used for this analysis.

Mean water (g), protein (g), fat (g) and ash (g) content of the carcass and legs obtained for the five pens in 10 samplings were used to describe the deposition of nutrients by fitting two nonlinear growth models (Gompertz and logistic), expressed in weight (g) as a function of age (days). The following models were adopted to describe nutrient deposition:

Gompertz (WINSOR, 1932):

$$Wt = Wm\, e^{-e^{-b\,(t-t^{*})}};$$

and logistic (REED and PEARL, 1927):

$$Wt = Wm \,/\, 1 + e^{-b\,(t-t^{*})};$$

where: Wt is the weight (body component) (g) at time t, expressed as a function of Wm; Wm is the weight (g) at maturity; b is the rate of deposition (g day^{-1}); t* is the time (days) when the maximum rate of deposition is attained, and t is time (days).

Nutrient deposition (g day^{-1}) as a function of time (t) was calculated by derivation of the Gompertz and logistic equations, respectively:

$$dWt'/dt = Wm\, b\, e^{-b\,(t-t^{*}) - e^{-b\,(t-t^{*})}};$$

and

$$dWt'/dt = b(Wt^{2}/Wm)e^{-b(t-t^{*})}.$$

The following equations were used to describe protein and fat intake: Gompertz (WINSOR, 1932):

$$It = Im\, e^{-e^{-b\,(t-t^{*})}};$$

and logistic (REED and PEARL, 1927):

$$It = Im \,/\, (1 + e^{-b\,(t-t^{*})});$$

where: It is the dietary intake of protein or fat (g) by the animal at time t, expressed as a function of Im; Im is the intake of protein or fat (g) at maturity of the animal; b is the rate of intake (g day^{-1}); t^{*} is the time (days) when the maximum rate of intake is attained, and t is time (days). On the basis of the parameters of the estimated equations, daily protein and fat intake (g day^{-1}) was calculated as a function of time (t) by derivation of the equations.

The parameters of the nonlinear equations were estimated using the NLIN procedure of the SAS program (SAS, 2008). The parameter estimates were obtained by a modified iterative Gauss-Newton method developed by HARTLEY (1961) for nonlinear models.

The following criteria were adopted to evaluate the goodness-of-fit of the models: residual mean square (RMS), Akaike information criterion (AIC) (AKAIKE, 1974), and mean absolute deviation of residuals (MAD) (SARMENTO *et al.*, 2006). The lower the value of MAD, the better the fit of the model.

The weights found for protein deposition in the carcass and protein intake were used to calculate the protein efficiency of the diet using the following linear equation: Y = A X + B, where the Y-axis = protein deposited in the carcass (g), X-axis = protein intake (g), A = slope of the linear regression line corresponding to the efficiency of protein utilization in %, and B = a constant corresponding to the intercept of the line with the vertical axis. The same equation was applied to determine the efficiency of fat intake by the animals. The PROC REG procedure (p = 0.05) of the SAS program (SAS, 2008) was used for this analysis.

RESULTS

The mean maximum and minimum ambient temperatures inside the rearing facility were 33.35 ± 3.20 °C and 21.26 ± 1.69 °C, respectively. The variation between the maximum and minimum temperature of the water was 30.1 ± 1.80 °C, where the maximum temperature was 30.95 ± 0.62 °C and the minimum 27.94 ± 1.88 °C. The average values of pH, dissolved oxygen and electrical conductivity of the water was 7.90 ± 0.10, 5.10 ± 0.30 mg L^{-1} and 201,64 ± 0,3 µS cm^{-1}, respectively.

A quadratic effect (p<0.05) was observed for moisture (Figure 1a), fat (Figure 1c) and crude energy (Figure 1i) content of the frog during the fattening phase as a function of time in days (Figure 1a-d). There was a linear effect for protein (p<0.05) (Figure 1b) and no effect of linear regression was observed for ashes (p>0.05) (Figure 1d). These results demonstrate the occurrence of quantitative changes in body nutrient composition during the fattening period.

The average centesimal composition of frog legs during the fattening phase was 79.29% water, 15.83% protein, 1.74% fat, and 2.99% ashes. The water content of legs decreased over the period analyzed (Figure 1e) and protein content increased linearly (Figure 1f). No effect of linear regression was observed for fat or ashes (Figures 1g and 1h).

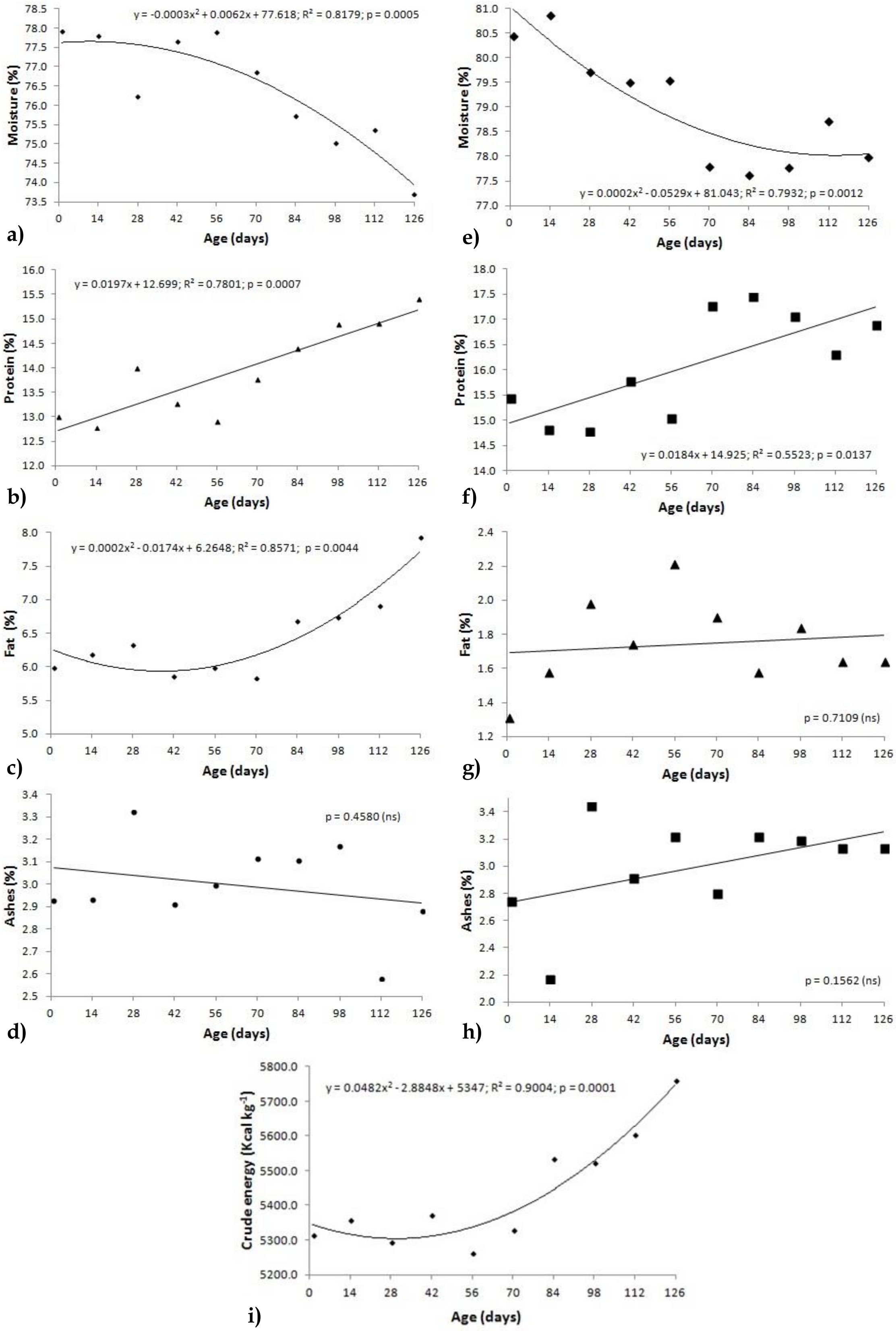

Figure 1. Body composition of bullfrogs during the fattening phase: moisture (a), protein (b), fat (c), and ashes (d). Composition of the legs: moisture (e), protein (f), fat (g), and ashes (h). Crude energy content of the bullfrog carcass during the fattening phase (i).

The lowest, and consequently the best, values of the model selection criteria (RMS, MAD and AIC) were obtained for the logistic model to describe the deposition of nutrients in the carcass and legs of bullfrogs, as well as protein and fat intake (Table 1).

The values of Wm, Im and t* estimated with the Gompertz model for dietary protein and fat intake and nutrient deposition in the carcass and legs of bullfrogs during the fattening phase may have been overestimated (Table 1), For example, dietary protein intake at maturity (Im) estimated with the logistic model was 137.4 g, whereas a value of 394.2 g was obtained with the Gompertz model (Table 1).

The values of t* estimated with the logistic model were 107.5 days for protein and fat intake, 106.1 days for water deposition, 113.5 days for protein deposition, 124.4 days for fat deposition, and 99.69 days for ash deposition in the carcass of bullfrogs. These values were 111.1 days for water, 104.0 days for protein, 86.91 days for fat, and 119.9 days for ash deposition in the legs. All values were within the 126 days of the experiment (Table 1).

Table 1. Estimates of the model parameters for cumulative dietary protein and fat intake and nutrient deposition in the carcass and legs of bullfrogs obtained with the Gompertz and logistic models.

Model	***Im* (g)**	***b* (g day^{-1})**	***t** (day)**	**RMS**	**MAD**	**AIC**
			Dietary protein intake			
Gompertz	394.2 ± 94.9	0.010 ± 0.001	156.7 ± 23.1	2.083	0.794	2.100
Logistic	137.4 ± 12.4	0.034 ± 0.002	107.5 ± 5.8	2.077	0.041	2.005
			Dietary fat intake			
Gompertz	51.8 ± 14.9	0.010 ± 0.001	156.7 ± 23.1	0.239	0.810	1.334
Logistic	16.9 ± 1.8	0.034 ± 0.002	107.5 ± 5.8	0.232	0.020	1.329
			Nutrient deposition in the carcass			
	***Wm* (g)**	***b* (g day^{-1})**	***t** (day)**	**RMS**	**MAD**	**AIC**
			Water			
Gompertz	653.3 ± 218.5	0.009 ± 0.002	162.0 ± 30.6	8.976	0.040	2.708
Logistic	244.3 ± 23.1	0.031 ± 0.002	106.1 ± 6.5	6.112	0.017	2.500
			Protein			
Gompertz	217.4 ± 103.9	0.008 ± 0.005	201.8 ± 44.5	0.500	0.266	2.864
Logistic	55.2 ± 6.0	0.033 ± 0.002	113.5 ± 6.9	0.322	0.099	2.433
			Fat			
Gompertz	226.9 ± 49.7	0.007 ± 0.002	274.8 ± 39.2	0.094	0.200	2.930
Logistic	30.9 ± 4.6	0.031 ± 0.002	124.4 ± 9.1	0.069	0.008	2.663
			Ashes			
Gompertz	18.8 ± 6.5	0.011 ± 0.002	137.7 ± 30.2	0.074	0.995	0,010
Logistic	8.6 ± 0.9	0.032 ± 0.003	99.7 ± 7.8	0.074	0.995	0.007
			Nutrient deposition in the legs			
			Water			
Gompertz	261.6 ± 34.5	0.009 ± 0.002	187.0 ± 0.8	0.986	0.014	1.512
Logistic	77.63 ± 8.5	0.032 ± 0.002	111.1 ± 7.2	0.650	0.009	1.423
			Protein			
Gompertz	34.7 ± 10.1	0.011 ± 0.002	145.6 ± 23.7	0.046	0.018	1.453
Logistic	14.5 ± 1.3	0.035 ± 0.002	104.0 ± 5.6	0.045	0.015	1.299
			Fat			
Gompertz	2.2 ± 0.4	0.015 ± 0.002	95.8 ± 12.9	0.003	0.003	1.556
Logistic	1.4 ± 0.1	0.038 ± 0.003	86.9 ± 4.6	0.002	0.003	1.456
			Ashes			
Gompertz	17.8 ± 3.8	0.008 ± 0.002	227.5 ± 33.9	0.002	0.035	1.006
Logistic	3.7 ± 0.6	0.032 ± 0.003	119.9 ± 11.1	0.002	0.031	1.003

Im = intake (g) at maturity; Wm = nutrient weight (g) at maturity; b = intake or deposition rate (g day^{-1}); t = time (days) when the maximum rate of intake or deposition is attained.*

The intake rate (b) estimated with the logistic model for protein and fat intake was 0.034 g day^{-1}, values similar to the deposition rates of water (0.031 g day^{-1}), protein (0.033 g day^{-1}), fat (0.031 g day^{-1}) and ashes (0.032 g day^{-1}) in the carcass of bullfrogs (Table 1).

The curves estimating protein and fat intake during the fattening period of bullfrogs obtained with the two models were different after day 42 of the experiment. In the logistic model, observed values were closer to the estimated values (Figure 2a, b), with this model being more adequate to estimate daily protein and fat intake (Figure 2c, d).

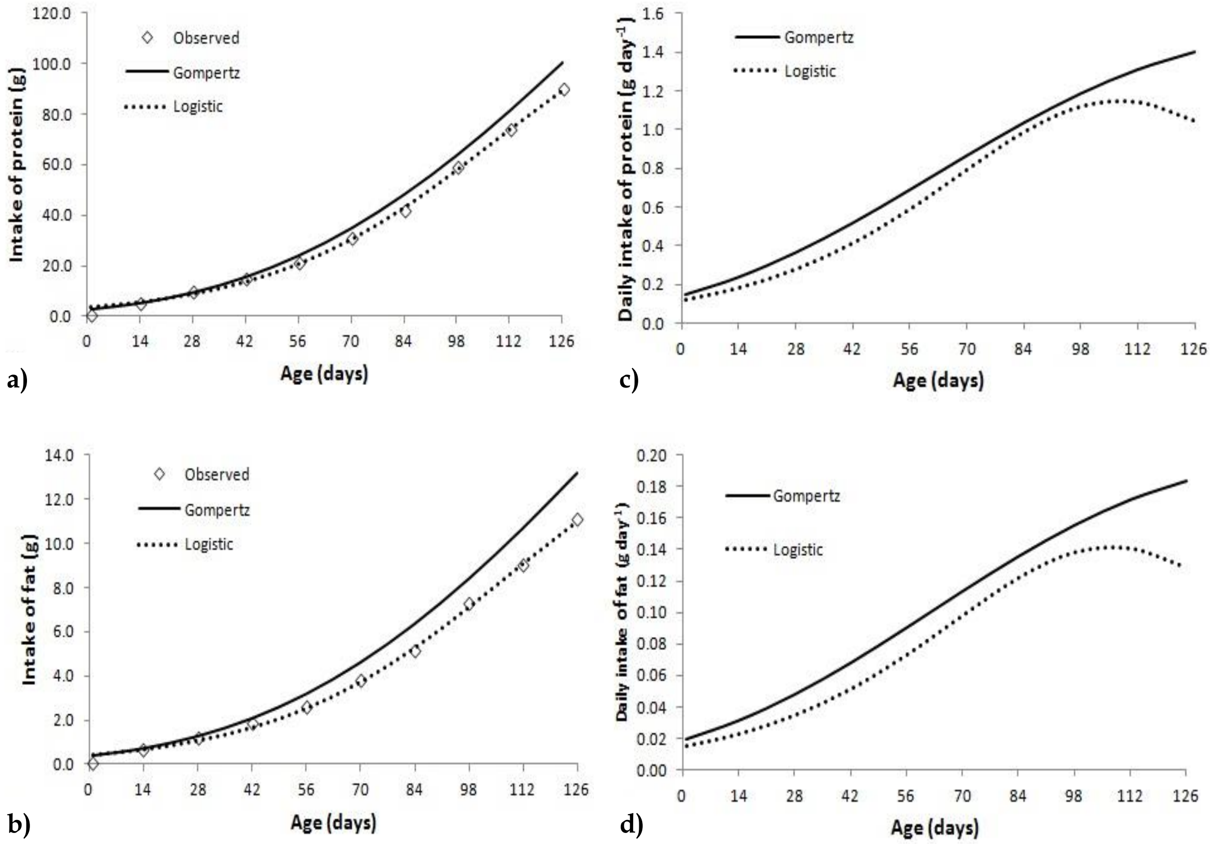

Figure 2. Gompertz and logistic curves to describe the intake of protein (a) and fat (b) and the respective daily intake (c, d) by bullfrogs during the fattening phase.

The estimated values of nutrient deposition in the carcass (Figure 3a-d) and legs (Figure 4a-d) of bullfrogs were close to the observed values. However, the curves for daily nutrient deposition obtained with the two models differed at the end of the experimental period, with the logistic curve showing a decline and the Gompertz curve exhibiting a general increase (Figures 3e-h; 4e-h).

Analysis of the relationship between protein deposition in the carcass and dietary protein intake showed low efficiency of protein utilization (36.76%) in bullfrogs fed a commercial diet during the fattening phase (Figure 5a). In contrast, analysis of the relationship between fat deposition and dietary fat intake showed an efficiency of 140.9% (Figure 5b).

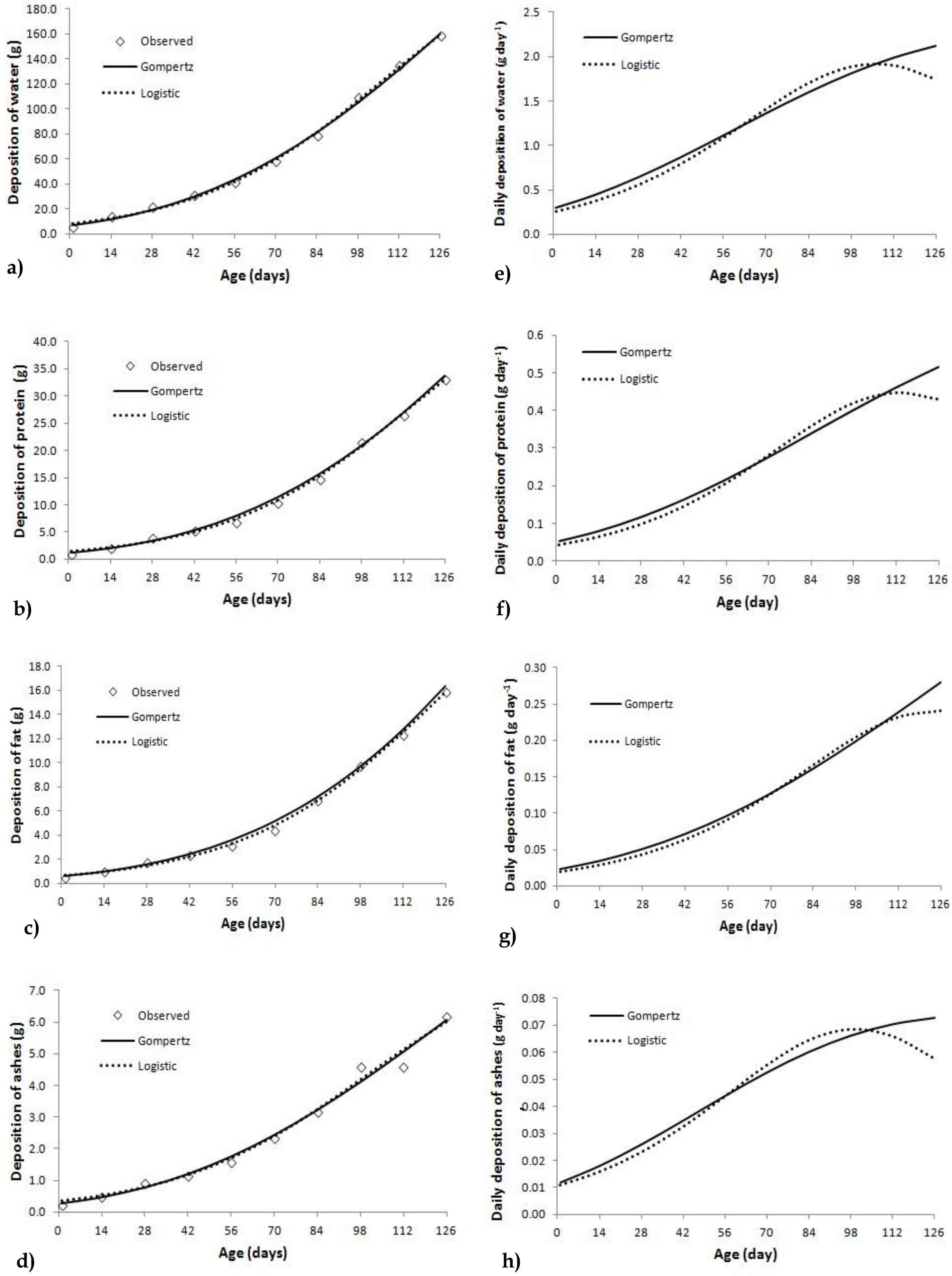

Figure 3. Gompertz and logistic curves to describe the deposition of nutrients in the bullfrog carcass during the fattening phase (a-d) and daily deposition (e-h).

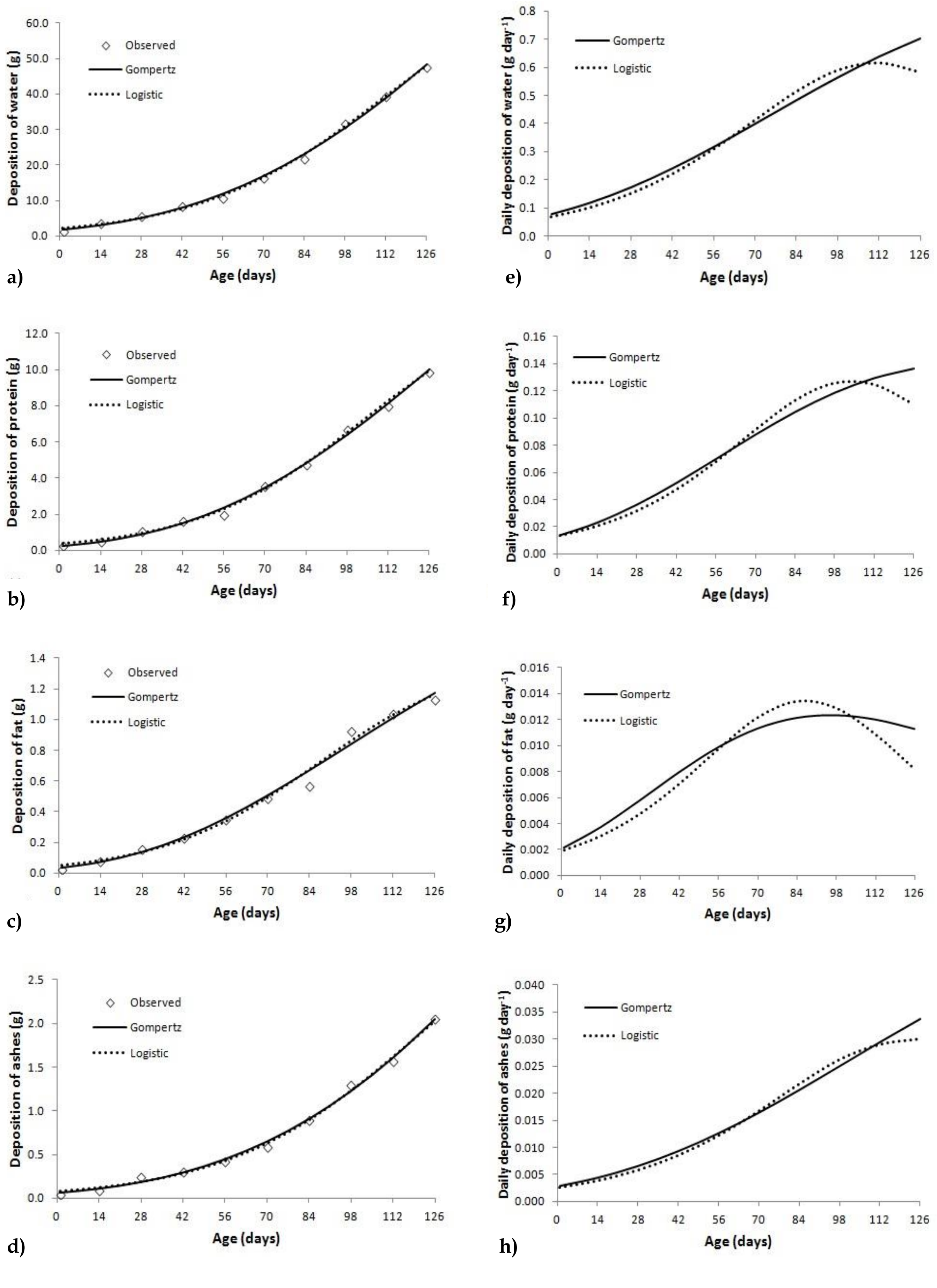

Figure 4. Gompertz and logistic curves to describe the deposition of nutrients in the legs of bullfrogs during the fattening phase (a-d) and daily deposition (e-h).

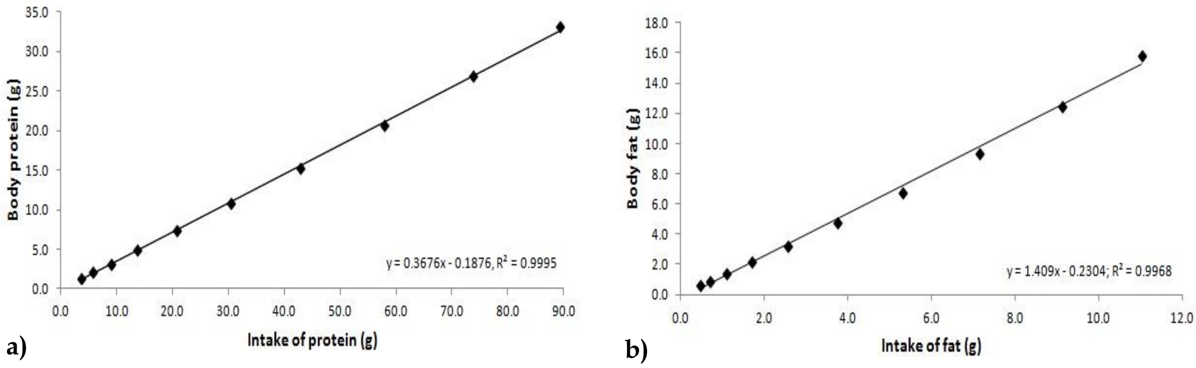

Figure 5. Efficiency of protein (a) and fat (b) utilization from the commercial diet fed to bullfrogs during the fattening phase.

DISCUSSION

Temperatures above 35 °C and below 15 °C, which could affect the growth of bullfrogs, were not observed. Temperature is a major factor interfering with the growth of bullfrogs since it directly influences the metabolism of the animal (PETERSEN and GLEESON, 2011). The values of pH, dissolved oxygen and electrical conductivity of the water were adequate for bullfrogs (BORGES *et al*., 2012).

Alterations were observed in most body and leg (muscles and bones) nutrients of bullfrogs during the fattening period. MELLO *et al*. (2006) found 79.2% water, 15.9% protein, 0.2% fat and 1.2% ashes in leg muscle, and 79.2% water, 15.7% protein, 0.2% fat and 0.9% ashes in the back of bullfrogs. ASSIS *et al*. (2009) observed 75.0% water, 23.4% protein, 0.16% fat and 1.17% ashes in leg and back muscles of bullfrogs. NÓBREGA *et al*. (2007) reported values of 74.1% water, 19.4% protein, 0.6% fat and 1.0% ashes for leg muscles of bullfrogs.

These differences in body composition are related to numerous factors. GONÇALVES *et al*. (2012) observed an influence of different oil sources on protein and fat content in lambari (*Astyanax altiparanae*). Comparison of fillet composition between two genetic groups of tilapia showed differences in protein and fat content (LUGO *et al*., 2003). Rainbow trout (*Oncorhynchus mykiss*) fed three times per day to satiation presented higher fat content of fillet and lower moisture content than animals fed once a day to satiation (HAFS *et al*., 2012).

The ash content of bullfrog legs observed in the present study was higher than that reported in the literature, probably because the samples consisted of meat and bone which contain large amount of minerals.

The best values of the model selection criteria were observed for the logistic model. Although overestimating the initial values for all variables studied, the logistic model was considered the best model to describe protein and fat intake and nutrient deposition in the carcass and legs of bullfrogs during the fattening phase. When the same models were compared to estimate the dynamics of in vitro ruminal fermentation from babassu (*Orbignya martiana*) meal and pie, the logistic model also overestimated initial values, but showed the best fit according to the same model selection criteria (FARIAS *et al*., 2011). Taken together, the results suggest that the logistic model tends to overestimate initial values, but approaches expected values over time, showing an excellent fit.

The values of Im and Wm estimated with the Gompertz model for protein and fat intake and for body and leg composition during the fattening phase were high. These high values may be adequate if the objective is to describe the growth of the animal throughout life, but studies with this objective need to be conducted. A frequent problem associated with growth curves is the lack of fit between values estimated by the parameters of the equation and values observed for the species (MURUYAMA *et al*., 2001).

Among five models studied (Gompertz, Brody, Richards, Von Bertalanffy, logistic), the logistic model was also found to be the most versatile to fit the growth of shrimp, pepper frog, rabbits, chickens, goats, sheep, pigs, and cattle (FREITAS, 2005). In contrast, RODRIGUES *et al*. (2007) selected the Gompertz and logistic models to estimate the weight gain of bullfrogs reared in mini-paddock pens. Thus, numerous factors (genetic diet, temperature, and management) may have influenced the dataset so that the fit of the Gompertz model was not satisfactory in the present study.

The first description of nutrient deposition in the bullfrog body during the fattening phase is important since body weight alone is not sufficient to determine the nutritional requirements of animals. According to NEME *et al*. (2006), body composition should be evaluated to improve feeding programs, and can also be used to determine the protein and energy requirements of animals (TRUNG *et al*., 2011).

The values of t* obtained with the logistic model for water and protein deposition in the bullfrog carcass were similar (106.1 and 113.5 days, respectively). This physiological process was observed in tadpoles of the same species, which start to retain more water during this phase of life because of higher protein synthesis (MANSANO *et al*., 2013). With respect to water and protein deposition in the legs of bullfrogs, the time of maximum deposition rates was 111.1 and 104.0 days respectively.

With respect to fat deposition in the bullfrog carcass, t* obtained with the logistic model occurred later (124.4 days) when compared to the other nutrients. This finding can be explained by the fact that the animal first grows in body structure and then accumulates fat in the fat bodies for reproduction and periods of hibernation (PEREIRA *et al*., 2011). In addition, crude energy increased in the body of bullfrogs as a function of time, mainly due to an increase in fat and protein deposition over time (Figure 1i). AMRKOLAIE *et al*. (2012) also observed an increase in fat deposition in growing sturgeon.

In frog legs, the maximum rate of fat deposition (t*) occurred at 86.9 days, with a daily deposition rate (b) of 0.038 g day^{-1}, demonstrating differences in fat deposition between the body and legs of bullfrogs. For the other nutrients, the rate of deposition (b) was similar in the carcass (0.031 g day^{-1} for water, 0.033 g day^{-1} for protein, and 0.032 g day^{-1} for ashes) and legs of bullfrogs (0.032, 0.035 and 0.03 g day^{-1}, respectively).

The maximum rate of ash deposition (t*) in bullfrogs during the fattening phase was observed at 99.7 days and occurred earlier than that found for the other nutrients, probably because of the formation of bones and structural tissues in the animals. However, the maximum rate of ash deposition occurred at 119.9 days in the legs, the latest time of all nutrients. This finding might be explained by the calcification of hypertrophic cartilage and deposition of trabecular bone which are late events and do not play an important role in the development and growth of long bones in the bullfrog. However, as the animals grow and gain weight, bone support becomes necessary (FELISBINO and CARVALHO, 2001). In addition, ashes deposited in the legs accounted for 43% of all ashes deposited in the bullfrog carcass. This finding indicates the need for further research on minerals. Protein deposition in the legs accounted for 26.3% of deposition of this nutrient in the carcass, water for 31.8%, and fat for only 0.45%. These results highlight the low fat content of bullfrog legs, a fact that may attract consumers who seek healthy foods.

The low protein efficiency (36.76%) of the commercial carnivorous fish diet fed to bullfrogs shows that dietary protein is not efficiently utilized by the animals, with major loss of a nutrient of high commercial value to the environment which is important for the animal (AMRKOLAIE, 2011; BORGES *et al*., 2012). In contrast, the high efficiency of fat utilization (140.9%) indicates the deposition of fat of non-dietary origin. In this case, excess dietary protein was probably diverted to the citric acid cycle in adipose tissue and transformed into and accumulated as fatty acids.

Among the possible ingredients used in the commercial diet, such as corn, wheat meal, blood meal, meat and bone meal and feather meal showed low digestibility of the protein fraction by the bullfrog (MANSANO, 2015), therefore enhances the low protein deposition efficiency in comparison with the quality of protein consumed by the frog.

According to AMRKOLAIE *et al*. (2012), the low protein efficiency observed for *Huso huso* despite its rapid growth may be explained by the high cost of urea excretion and lipogenesis. The protein for maintenance and growth of beluga sturgeon may indicate that the animal is using protein as an energy source to satisfy its large energy requirements for growth. Therefore, dietary energy should be derived from a non-protein source.

One approach to increase the protein efficiency of frog diets is the use of ingredients with greater digestibility. Another possibility is animal improvement. In this respect, studies using different fish lineages have shown differences in nutrient deposition over time and in the efficiency of utilization of diet components (LIEBERT *et al*., 2006).

The present study demonstrates the need for further investigation of the digestibility of foods and of the requirements of protein, energy, mineral and vitamins of bullfrogs in order to obtain data for the elaboration of a diet for this species (SEIXAS FILHO *et al*., 2009).

CONCLUSIONS

The curves of dietary protein and fat intake and nutrient deposition in the body and legs of bullfrogs, which were best described by the logistic model, illustrated the course of deposition of each nutrient. The commercial diet showed low protein efficiency (36.76%) and high efficiency of fat utilization (140.9%) in bullfrogs, indicating the need to develop an ideal diet for these animals.

ACKNOWLEDGEMENTS

We thank the state funding agency Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support (Grant 11/50865-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a doctoral fellowship to the first author. We also thank the technician Márcio Roberto Reche for help with the study.

REFERENCES

AKAIKE, H. 1974 A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, *19*(1): 716-723.

AMRKOLAIE, A.K. 2011 Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. *Reviews in Aquaculture*, *3*(1): 19-26.

AMRKOLAIE, A.K.; YANSARI, A.T.; KHALESI, M.K. 2012 Calculation of protein and energy requirements in beluga sturgeon (*Huso huso*) using a factorial approach. *Journal Animal Physiology and Animal Nutrition*, *96*(1): 1-10.

ASSIS, M.F.; FRANCO, M.L.R.S.; STÉFANI, M.V.; FRANCO, N.P.; GODOY, L.C.; OLIVEIRA, A.C.; VISENTAINER, J.V.; SILVA, A.F.; HOCH, A.L.V. 2009 Efeito do alecrim na defumação da carne de rã (*Rana catesbeiana*): características sensoriais, composição e rendimento. *Ciência e Tecnologia dos Alimentos*, *29*(3): 553-556.

BORGES, F.F.; AMARAL, L.A.; STEFANI, M.V. 2012 Characterization of effluents from bullfrog (*Lithobates catesbeianus*, Shaw, 1802) grow-out ponds. *Acta Limnologica Brasiliensia*, *24*(1): 160-166.

CASALI, A.P.; MOURA, O.M.; LIMA, S.L.; SILVA, J.H.V. 2005 Avaliação de rações comerciais nas fases de crescimento e terminação da recria de rã-touro. *Boletim do Instituto de Pesca*, *31*(1): 37-46.

CASAS, G.A.; RODRÍGUEZ, D.; AFANADOR, G. 2010 Propiedades matemáticas del modelo de Gompertz y su aplicación al crecimiento de los cerdos. *Revista Colombiana de Ciencias Pecuarias*, *23*(2): 349-358.

DUMAS, A.; FRANCE, J.; BUREAU, D. 2010 Modelling growth and body composition in fish nutrition: Where have we been and where are we going? *Aquaculture Research*, *41*(2): 161-181.

ETHERIDGE, R.D.; PESTI, G.M.; FOSTER, E.H. 1998 A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. *Animal Feed Science and Technology*, *73*(1): 21-28.

FARIAS, L.N.; VASCONCELOS, V.R.; CARVALHO, V.R.; SARMENTO, J.L.R. 2011 Avaliação dos modelos logístico bicompartimental e de Gompertz na estimativa da dinâmica de fermentação ruminal *in vitro* do farelo e da torta de babaçu (*Orbignya martiana*). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *63*(1): 136-142.

FELISBINO, S.L. and CARVALHO, H.F. 2001 Growth cartilage calcification and formation of bone trabeculae are late and dissociated events in the endochondral ossification of *Rana catesbeiana*. *Cell Tissue Research*, *306*(2): 319-323.

FENERICK JR, J. and STÉFANI, M.V. 2005 Desempenho e parâmetros metabólicos de rã-touro, *Rana catesbeiana*, alimentada com diferentes rações comerciais. *Acta Scientiarium. Animal Science*, *27*(2): 377-383.

FREITAS, A.R. 2005. Curvas de Crescimento na Produção Animal. *Revista Brasileira de Zootecnia*, *34*(4): 786-795.

GONÇALVES, L.U.; FERROLI, F.; VIEGAS, E.M.M. 2012 Effect of the inclusion of fish residue oils in diets on the fatty acid profile of muscles of males and females lambari (*Astyanax altiparanae*). *Revista Brasileira de Zootecnia*, *41*(8): 1967-1974.

HAFS, A.W.; MAZIK, P.M.; KENNEY, P.B.; SILVERSTEIN, J.T. 2012 Impact of carbon dioxide level, water velocity, strain, and feeding regimen on growth and fillet attributes of cultured rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *350*(1): 46-53.

HARTLEY, H.O. 1961 The modified Gauss Newton method for the fitting of non linear regression functions by least squares. *Technometrics*, *3*(2): 269-280.

HUA, K.; BIRKETT, S.; DE LANGE, C.F.M.; BUREAU, D.P. 2010 Adaptation of a non-ruminant nutrient-based growth model to rainbow trout (*Oncorhynchus mykiss* Walbaum). *Journal of Agricultural Science*, *148*(1): 19-27.

LIEBERT, F.; SÜNDER, A.; MOHAMED, K. 2006 Assessment of nitrogen maintenance requirement and potential for protein deposition in juvenile Tilapia genotypes by application of an exponential nitrogen utilization model. *Aquaculture*, *78*(4): 812-821.

LIMA, S.L. 1997 *Criação de rãs (Sistema Anfrigranja)*. Viçosa: CPT, Manual técnico (08). 48p.

LUGO, M.G.; ALVAREZ, I.G.; NOVOA, M.A.O.; CÓRDOVA, G.M. 2003 Comparison of growth, fillet yield and proximate composition between Stirling Nile tilapia (wild type) (*Oreochromis niloticus*, Linnaeus) and red hybrid tilapia (Florida red tilapia x Stirling red *O. niloticus*) males. *Aquaculture Research*, *34*(2): 1023-1028.

MANSANO, C.F.M. 2015 *Digestibilidade e exigência de aminoácidos para rã-touro*. Jaboticabal. 115f. (Tese de Doutorado. Centro de Aquicultura (CAUNESP), Universidade Estadual Paulista - UNESP).

MANSANO, C.F.M.; DE 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.

MARCATO, S.M.; SAKOMURA, N.K.; MUNARI, D.P.; FERNANDES, J.B.K.; KAWAUCHI, I.M.; BONATO, M.A. 2008 Growth and body nutrient deposition of two broiler commercial genetic lines. *Brazilian Journal of Poultry Science*, *10*(3): 117-123.

MELLO, S.C.R.P.; SILVA, L.E.; MANO, S.; FRANCO, R.M. 2006 Avaliação bacteriológica e físico-química das carnes do dorso e coxa de rã (*Rana catesbeiana*) processadas em matadouro comercial. *Revista Brasileira de Ciências Veterinárias*, *13*(1): 151-154.

MURUYAMA, K.; VINYARD, B.; AKBAR, M.K.; SHAFER, D.J.; TURK, C.M. 2001 Growth curve analyses in selected duck lines. *British Poultry Science*, *42*(1): 574-582.

NEME, R.; SAKOMURA, N.K.; FUKAYAMA, E.H.; FREITAS, E.R.; FIALHO, F.B.; RESENDE, K.T.; FERNANDES, J.B.K. 2006 Curvas de crescimento e de deposição dos componentes corporais em aves de postura de diferentes linhagens. *Revista Brasileira de Zootecnia*, *35*(6): 1091-1100.

NEVEU, A. 2009 Suitability of European Green frogs for intensive culture: comparison between different phenotypes of the esculenta hybridogenetic complex. *Aquaculture*, *295*(5): 30-37.

NÓBREGA, I.C.C.; ATAÍDE, C.S.; MOURA, O.M.; LIVERA, A.V.; MENEZES, P.H. 2007 Volatile constituents of cooked bullfrog (*Rana catesbeiana*) legs. *Food Chemistry*, *102*(3): 186-191.

OLVERA-NOVOA, M.A.; ONTIVEROS-ESCUTIA, V.M.; FLORES-NAVA, A. 2007 Optimum protein level for growth in juvenile bullfrog (*Rana catesbeiana*, Shaw, 1802). *Aquaculture*, *266*(10): 191-199.

PEREIRA, M.M.; RIBEIRO FILHO, O.P.; TRONI, A.R.; TAKAMURA, A.E.; MANSANO, C.F.M.; NASCIMENTO, F.B.; CAMARGO FILHO, C.B. 2011 Tecido hepático e corpo adiposo de rã-touro (*Lithobates catesbeianus*) submetido a diferentes fotoperíodos. *Acta Veterinaria Brasilica*, *5*(1): 76-80.

PETERSEN, A.M. and GLEESON, T.T. 2011 Acclimation temperature affects the metabolic response of amphibians skeletal muscle to insulin. *Comparative Biochemistry and Phisiology*, *160*(3): 72-80.

REED, L.J. and PEARL, R. 1927 On the summation of logistic curves. *Journal of the Royal Statistical Society*, *90*(1): 729-746.

RODRIGUES, M.L.; LIMA, S.L.; MOURA, O.M.; AGOSTINHO. C.A.; SILVA. J.H.V.; CRUZ, G.R.B.; CAMPOS, V.M.; CASALI, A.P.; MENDES, R.R.B.; ALBUQUERQUE, A.G. 2007 Curva de crescimento em rã-touro na fase de recria. *Archivos de Zootecnia*, *56*(1): 125-136.

SARMENTO, J.LR.; REGAZZI, A.J.; SOUZA, W.H.; TORRES, R.A.; BREDA, F.C.; MENEZES, G.R.O. 2006 Analysis of the growth curve of Santa Ines sheep. *Revista Brasileira de Zootecnia*, *35*(3): 435-442.

SAS Institute. 2008 SAS/STAT 9.2. *User's guide*. SAS Institute Inc, Cary, NC. p.4264-4286.

SEIXAS FILHO, J.T.; HIPOLITO, M.; MARTINS, A.M.C.R.P.F.; RODRIGUES, E.; CASTAGNA, A.A.; MELLO, S.C.R.P. 2009 Histopathological alterations in bullfrog juveniles fed commercial rations of different crude protein levels. *Revista Brasileira de Zootecnia*, *39*(6): 2306-2310.

SILVA, D.J. and QUEIROZ, A.C. 2006 *Análise de alimentos métodos químicos e biológicos*. 3rd ed. Viçosa, MG: Editora UFV. 235p.

TOKUR, B.; GÜRBÜZ, D.; ÖZYURT, G. 2008 Nutricional composition of frog (*Rana esculanta*) waste meal. *Bioresource Technology*, *99*(6): 1332-1338.

TRUNG, D.V.; DIU, N.T.; HAO, N.T.; GLENCROSS, B. 2011 Development of a nutritional model to define the energy and protein requirements of tilapia, *Oreochromis niloticus*. *Aquaculture*, *320*(1): 69-75.

TURNIPSEED, S.B.; CLARK, S.B.; STOREY, J.M.; CARR, J.R. 2012 Analysis of veterinary drug residues in frog legs and other aquacultured species using liquid chromatography quadrupole time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry*, *60*(2): 4430-4439.

WINSOR, C.P. 1932 The Gompertz curve as a growth curve. *Proceedings of the National Academy of Sciences of the United States of America*, *18*(1): 1-8.