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Determination of digestible lysine and estimation of essential amino acid requirements for bullfrogs

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ABSTRACT

The traditional method to determine the nutritional requirements of animals is based on individual dose–response experiments that are time consuming and costly. One alternative to establish the dietary protein profile is the use of the ideal ratio between essential amino acids and digestible lysine to estimate essential amino acid requirements. The objective of this study was to determine the digestible lysine requirement of bullfrogs and to estimate essential amino acid requirements based on the ideal ratio. The experimental design was completely randomized and consisted of five treatments and three replicates. Six hundred froglets with an initial mean weight of 51.53 ± 1.91 g, identified through an implanted transponder, were used. The experimental diet (30.42% digestible protein) was supplemented with five levels of lysine HCl (0, 0.7, 1.4, 2.1, and 2.8%), so that the diets contained 1.38, 2.11, 2.85, 3.65 and 4.39% digestible lysine. The performance variables were feed intake, weight gain, feed conversion, specific growth rate, protein efficiency ratio, protein retention efficiency, and body protein and lysine deposition. The optimum level of digestible lysine was obtained at the intersection of the ascending line with the response plateau. Weight gain increased until reaching 222.7 g at 2.23% digestible lysine in the diet. The best feed conversion (1.4 g/g) and body protein (48.28 g) and lysine (4.07 g) deposition were observed at 2.29%, 2.33% and 2.39% digestible lysine, respectively. The digestible lysine requirement of bullfrogs is 2.71% of dry weight or 8.91% of dietary digestible protein, a level that provides the highest protein retention efficiency. The requirements of the other digestible amino acids estimated based on the concept of the ideal ratio of essential amino acids are (of dry weight): 2.16% arginine; 0.94% histidine; 1.34% isoleucine, 2.39% leucine; 0.79% methionine; 1.31% phenylalanine; 1.34% threonine; 0.23% tryptophan; 1.58% valine; 0.36% cystine, and 1.07% tyrosine.

Statement of Relevance: The estimate requirement of digestible essential amino acid using the concept of the ideal ratio permits the elaboration of diets with an appropriate amino acid, maximizing growth, protein utilization efficiency and carcass quality for bullfrogs.

1. Introduction

Brazilian frog farming has a great potential as a producer of high-quality and low-fat animal protein. However, this is an area that needs to be better exploited through the use of tools that assist researchers, technicians and producers to maximize production and minimize costs within a productive (Dias et al., 2010) and sustainable process (Bosma and Verdegem, 2011), for a population that requires more food from fewer natural resources (Schneider et al., 2011). Within this context, it is first necessary to establish all nutritional requirements of the species studied. Several concepts exist for this purpose (Glencross et al., 2007).

The method traditionally used for the determination of amino acid requirements in aquatic organisms relies on individual dose–response experiments of each amino acid, which are time consuming and costly (Small and Soares, 1998). The concept of the ideal amino acid ratio is based on the balance of free or protein-bound amino acids readily available for digestion and metabolism to obtain the optimum productive performance (Sakomura and Rostagno, 2007). Theoretically, the ideal amino acid ratio should be identical to the amino acid profile of the body and the animal's needs for growth and maintenance of metabolic activities (Li et al., 2009). Thus, the amino acid profile of body protein is the concept most commonly used in studies to represent the amino acid requirements of growing animals (Kaushik, 1998; Kim and Lall,

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2000; Abimorad et al., 2010; Grisdale-Helland et al., 2011; Cao et al., 2012).

The advantage of this concept is that it can be easily adapted and modified to different situations, especially the formulation of nutritionally complete diets. The ideal ratios remain relatively stable, irrespective of the substitution of ingredients in the nutritional composition. Normally, the most limiting essential amino acid (EAA) is used to estimate the requirement of the other amino acids by means of the ideal EAA ratio of the body (Twibell et al., 2003; Wang et al., 2005).

Lysine has been used as the reference amino acid in almost all studies because it is the main amino acid for protein deposition in the animal and is necessary for metabolic interactions with other amino acids (Ball et al., 2007). In view of the important role of lysine as a reference for the absorption of other amino acids, its requirement should be defined as accurately as possible based on the concept of the ideal ratio of EAA in order to avoid biased estimates of the requirements of other amino acids.

In view of the above considerations, the objective of this study was to establish the digestible lysine requirement of bullfrogs (based on dose–response experiments) by evaluating productive performance and efficiency of nutrient utilization. Additionally, the nutritional requirements of other amino acids were estimated by determining the ideal ratio between EAA and the level of digestible lysine that provided the best performance results. The results of this study will provide the scientific basis for the formulation of adequate diets, increasing their assimilation and consequently reducing environmental impacts.

2. Material and methods

The experiment was conducted between December 2013 and March 2014 at the Frog Farming Sector and Laboratory of Nutrition of Aquatic Organisms, Aquaculture Center of Universidade Estadual Paulista (UNESP), Jaboticabal Campus.

All procedures were approved by the Ethics Committee on Animal Use of the School of Agricultural and Veterinary Sciences, UNESP (Protocol No. 011866/12), and were conducted according to the ethical principles adopted by the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal — COBEA).

2.1. Biological material and facilities

Six hundred froglets with an initial weight of 51.53 ± 1.91 g were randomly allocated to 15 experimental pens (3 m^2) in the fattening facility. The pens were equipped with linearly arranged vibrating feeders, shelters, and water troughs. All animals were identified by implantation of a bioglass-encapsulated transponder (Animal Tag, ISO FDX-B, 134.2 kHz) compatible with norms ISO 11784, 11,785 and NBR 14766, measuring 2.2 × 12.2 mm, according to the method of Mansano et al. (2013).

Water was supplied continuously from an artesian well at a temperature of about 28 °C. The pens were cleaned daily and the water troughs were emptied, unconsumed feed was removed, and the water was exchanged. The temperature and humidity in the fattening facility

Table 1
Formula and nutritional composition of the diet used for bullfrogs.

	Level of digestible lysine (%)				
	1.38	2.11	2.85	3.65	4.39
Ingredient (%)					
Corn grain	23.00	23.00	23.00	23.00	23.00
Corn gluten mean	20.00	20.00	20.00	20.00	20.00
Wheat bran	19.56	19.56	19.56	19.56	19.56
Hydrolyzed feather meal	15.03	15.03	15.03	15.03	15.03
Salmon by-product meal	10.00	10.00	10.00	10.00	10.00
Soybean meal	6.00	6.00	6.00	6.00	6.00
L-Lysine HCl — 78%	0.00	0.70	1.40	2.10	2.80
Glutamic acid — 99%	3.50	2.80	2.10	1.40	0.70
DL-methionine — 99%	0.46	0.46	0.46	0.46	0.46
L-Histidine — 99%	0.13	0.13	0.13	0.13	0.13
L-Tryptophan — 98%	0.03	0.03	0.03	0.03	0.03
Monopotassium phosphate	1.77	1.77	1.77	1.77	1.77
Mineral and vitamin supplement[a]	0.50	0.50	0.50	0.50	0.50
BHT[b]	0.02	0.02	0.02	0.02	0.02
Composition analyzed (dry matter basis)					
Crude protein (%)[c]	44.22	43.80	43.23	44.12	43.86
Digestible protein (%)	30.69	30.39	29.95	30.62	30.44
Crude ether extract (%)[d]	6.71	6.68	6.68	6.70	6.80
Crude and digestible essential amino acids (%)[e] (dry matter basis)					
Arginine	[f]2.73 (2.34)	2.66 (2.29)	2.66 (2.29)	2.69 (2.32)	2.63 (2.27)
Histidine	0.95 (0.80)	0.96 (0.80)	0.96 (0.80)	0.96 (0.80)	0.94 (0.79)
Isoleucine	2.07 (1.69)	2.04 (1.67)	2.04 (1.66)	1.99 (1.63)	2.00 (1.64)
Leucine	5.32 (4.39)	5.26 (4.35)	5.17 (4.27)	5.15 (4.25)	5.15 (4.25)
Lysine	1.70 (1.38)	2.45 (2.11)	3.19 (2.85)	3.99 (3.65)	4.74 (4.39)
Methionine	1.49 (1.32)	1.48 (1.31)	1.48 (1.31)	1.47 (1.30)	1.49 (1.32)
Phenylalanine	2.41 (1.65)	2.37 (1.62)	2.38 (1.62)	2.39 (1.69)	2.39 (1.68)
Threonine	1.90 (1.35)	1.92 (1.36)	1.86 (1.32)	1.89 (1.34)	1.89 (1.34)
Tryptophan	0.33 (0.26)	0.32 (0.25)	0.31 (0.24)	0.30 (0.24)	0.32 (0.25)
Valine	2.98 (2.49)	2.95 (2.46)	2.92 (2.42)	2.96 (2.47)	2.95 (2.46)

[a] Moisture (%) 2.0; ashes (%) 71.6442; choline (mg/kg) 30,000; magnesium (%) 0.0085; sulfur (%) 1.1589; iron (mg/kg) 25,714; copper (mg/kg) 1960; manganese (mg/kg) 13,345; zinc (mg/kg) 30,000; iodine (mg/kg) 939; selenium (mg/kg) 30; vitamin A (IU/kg) 600,000; vitamin D3 (IU/kg) 600,000; vitamin E (mg/kg) 12,000; vitamin K3 (mg/kg) 631; vitamin B1 (thiamin, mg/kg) 1176; vitamin B2 (riboflavin, mg/kg) 1536; vitamin B6 (pyridoxine, mg/kg) 1274; vitamin B12 (μg/kg) 4000; niacin (mg/kg) 19,800; vitamin B3 (pantothenic acid, mg/kg) 3920; folic acid (mg/kg) 192; biotin (mg/kg) 20; ascorbic acid (mg/kg) 40,250.

[b] Butylated hydroxytoluene.

[c] Method of Dumas in a Leco 528 LC apparatus (Etheridge et al., 1998).

[d] Acid hydrolysis (AOAC, 1995).

[e] Acid hydrolysis and ion-exchange chromatography (HPLC).

[f] Crude AA (Digestible AA).

were measured daily with a digital thermo-hygrometer. The mean maximum and minimum temperature and relative humidity were, respectively, 31.6 ± 1.61 °C and 21.9 ± 1.14 °C and 80.4 ± 4.53% and 32.5 ± 10.12%. According to Braga and Lima (2001) and Figueiredo et al. (2001), the best productive performance of bullfrogs is obtained at a temperature of 25 to 30 °C, a value close to the mean temperatures observed in the present study.

2.2. Experimental diets

Protein, ether extract and digestible amino acid data were used for formulation of the diets (Mansano, 2015). For diet preparation, the ingredients were mixed and grounded using a hammer mill (Model 4, D'Andrea, Limeira, Brazil) fitted with a 0.8 mm screen sieve. Diets were extruded at a diameter of 4 to 6 mm, under identical processing conditions using a single-screw extruder (Mab 400S, Extrucenter, Monte Alto, Brazil) with an average extrusion capacity of 150 kg h^{-1}. The extruder pre-conditioning temperature was maintained above 90 °C. The water and steam additions, screw speed and food flux were adjusted according to the dietary formulations, and the extrusion temperature varied between 125 and 135 °C, and dried in a dryer at 105 °C temperature for 1 h.

For evaluation of the digestible lysine requirement of bullfrogs, an experimental diet containing 30.42% digestible protein (with the minimum lysine possible) was formulated and supplemented with five levels of lysine HCl (0, 0.7, 1.4, 2.1, and 2.8%) (Table 1). The ratio of the other amino acids was maintained according to the previously determined body amino acid profile of bullfrogs (3.69% arginine; 1.61% histidine; 2.29% isoleucine; 4.08% leucine; 4.63% lysine; 1.35% methionine; 2.24% phenylalanine; 2.28% threonine; 0.39% tryptophan; 2.70% valine).

2.3. Management and laboratory tests

The diets were offered *ad libitum* all the time in vibrating feeders. Leftovers were removed, stored in a freezer, and subsequently dried in a forced air circulation oven for calculation of the amount of ingested feed.

The animals were weighed on a precision scale (0.01 g) at the beginning and at the end of the experimental period (90 days) for determination of the performance variables (weight gain, feed intake, apparent feed conversion, specific growth rate, and protein efficiency ratio), crude protein and lysine deposition, and protein retention efficiency.

First, a sample of 20 animals representing the whole lot was obtained. At the end of the experiment, three animals per pen were sampled, transferred to polyethylene boxes with a small water layer, and fasted for 48 h. After this period, the animals were stunned by immersion in ice and the spine was sectioned. The animals were frozen for subsequent grinding in a meat grinder, lyophilized, ground again in a ball mill, and sent for determination of dry matter and crude protein.

For analysis of body nutrients, crude protein was determined by the method of Dumas in a Leco 528 LC apparatus (Etheridge et al., 1998). Dry matter was obtained in an oven at 105 °C for 12 h. The methods described by the AOAC (1995) were used for the analyses. Total amino acids were determined by acid hydrolysis and ion-exchange chromatography (HPLC) in the Laboratory of Protein Sources, Universidade Estadual de Campinas (UNICAMP) according to the method of White et al. (1986) and Hagen et al. (1989).

At the end of the study and after analysis of the data for determination of the most appropriate level of digestible lysine, the ratio of digestible protein to adequate digestible lysine was established for fattening bullfrogs. Additionally, the amino acid profile, called the "ideal EAA ratio", was determined based on the ratio of body amino acids of bullfrogs according to the method of Arai (1981).

2.4. Experimental design and statistical analysis

A completely randomized design consisting of five treatments and three replicates was used. For the evaluation of weight gain, each animal was considered one replicate. For evaluation of the other performance parameters and nutrient deposition, each pen was considered one replicate, for a total of three replicates per treatment. The values of the performance variables and body protein and lysine deposition were obtained at the intersection of the ascending line with the response plateau, determining the minimum digestible lysine level for each variable. These variables were obtained by the broken-line model using the SAS software (SAS Institute, 2008).

3. Results

With respect to productive performance, nutrient deposition and nutrient retention efficiency, the addition of lysine directly influenced ($P < 0.05$) the responses of the animals (Table 2).

The mean weight gain, specific growth rate, feed intake, apparent feed conversion, protein efficiency ratio, protein deposition, lysine deposition and protein retention efficiency were fitted with a broken-line model, in which the breakpoint of the line was considered the ideal level of digestible lysine (Table 3). The best weight gain (222.7 g) and feed conversion (1.40 g/g) of the animals estimated with the broken-line model were obtained at 2.23% and 2.29% digestible lysine, respectively. The highest specific growth rate (1.85%/day) was obtained at 2.21% digestible lysine in the diet (Table 3). The optimum

Table 2
Mean values of the productive performance variables, nutrient deposition and nutrient retention efficiency of bullfrogs fed diets containing different levels of digestible lysine.

	Dietary level of digestible lysine (%)					ANOVA *P*-value	F
	1.38	2.11	2.85	3.65	4.39		
Live weight gain (g)	142.34 ± 7.30	210.93 ± 12.08	217.77 ± 5.81	231.71 ± 5.24	218.50 ± 12.74	0.0001	29.68
Specific growth rate (%/day)	1.48 ± 0.02	1.81 ± 0.04	1.85 ± 0.06	1.91 ± 0.04	1.81 ± 0.07	0.0001	22.52
Feed intake (g)	235.26 ± 4.08	306.64 ± 13.03	306.99 ± 12.51	325.93 ± 12.76	304.60 ± 11.61	0.0001	19.09
Apparent feed conversion (g/g)	1.66 ± 0.08	1.45 ± 0.02	1.41 ± 0.06	1.41 ± 0.02	1.40 ± 0.04	0.0013	10.42
Protein efficiency ratio (g/g)	1.97 ± 0.10	2.27 ± 0.04	2.36 ± 0.11	2.28 ± 0.04	2.28 ± 0.04	0.0001	22.52
Protein deposition (g)	26.69 ± 1.25	43.18 ± 2.39	45.28 ± 0.68	52.26 ± 1.18	47.30 ± 2.87	0.0001	54.08
Lysine deposition (g)	2.16 ± 0.10	3.53 ± 0.20	3.86 ± 0.05	4.26 ± 0.11	4.09 ± 0.26	0.0001	53.85
Protein retention efficiency (%)	61.55 ± 3.13	70.04 ± 1.18	79.29 ± 3.89	70.15 ± 0.53	81.49 ± 1.19	0.0001	23.05

Values are means of three replicates ± standard deviation.
Live weight gain (g) = (final weight − initial weight).
Specific growth rate (%/day) = (((ln (final weight) − ln (initial weight))/(time (days))).
Apparent feed conversion (g/g) = ((feed intake)/(live weight gain)).
Protein efficiency ratio (g/g) = ((live weight gain)/(crude protein ingested)).
Nutrient deposition (g) = ((live weight gain × nutrient content in the body) / 100)).
Crude protein retention efficiency (%) = $(((CP_F \times W_F) - (CP_I \times W_I)) \times 100) / I_{CP})$)), where CP_F = final crude protein in the body; CP_I = initial crude protein in the body; I_{CP} = mean crude protein intake of the portion; W_I, W_F = mean initial and final live weight of the portion.

Table 3
Equations fitted with the broken-line model for the productive performance variables, nutrient deposition and nutrient retention efficiency of bullfrogs fed diets containing different levels of digestible lysine.

Variable	Optimum level of digestible lysine (%)	R^2
Live weight gain (g)	2.23	0.8997
y = 222.7 − 91.4489 (2.2283 − x)		
Specific growth rate (%/day)	2.21	0.8617
y = 1.8556 − 0.4356 (2.2122 − x)		
Feed intake (g)	2.16	0.8365
y = 312.50 − 95.1733 (2.1616 − x)		
Apparent feed conversion (g/g)	2.29	0.8047
y = 1.4044 − 0.2667 (2.2958 − x)		
Protein efficiency ratio (g/g)	2.19	0.7528
y = 2.3078 − 0.4000 (2.1944 − x)		
Protein deposition (g)	2.33	0.8901
y = 48.2789 − 22.0000 (2.3316 − x)		
Lysine deposition (g)	2.39	0.9288
y = 4.0689 − 2.3968 (2.3968 − x)		
Protein retention efficiency (%)	2.71	0.7499
y = 76.9756 − 11.3111 (2.7135 − x)		

level of digestible lysine in the diet was similar for maximum protein (48.28 g) and lysine deposition (4.07 g) in the animal's body and maximum protein retention (77.0%) (2.33, 2.39 and 2.71%, respectively). The best protein efficiency ratio (2.3 g/g) was observed at 2.19% digestible lysine in the diet (Table 3).

The optimum level of digestible lysine (2.71%) obtained with the equation for protein retention efficiency was used to calculate the ideal amino acid profile for bullfrogs. Table 4 shows the percentage of EAA in the body of bullfrogs, the EAA/ΣEAA ratio (Arai, 1981), and the ideal EAA profile for the species. To determine the EAA profile, the requirement of the other digestible essential amino acids was estimated based on the appropriate level of digestible lysine determined in this dose–response study.

The estimated EAA requirements for the diet of bullfrogs were: 2.16% arginine; 0.94% histidine; 1.34% isoleucine, 2.39% leucine; 2.71% lysine (determined in this study); 0.79% methionine; 1.31% phenylalanine; 1.34% threonine; 0.23% tryptophan, 1.58% valine, 0.36% cystine and 1.07% tyrosine (Table 4).

4. Discussion

Purified or semi-purified diets are used in most dose–response studies for determination of the amino acid requirements of aquatic organisms. These diets may negatively influence the growth of the animals because of a reduction in feed intake (Berge et al., 2002), especially when the diets are deficient in EAA, particularly lysine (Yamamoto et al., 2001; Dabrowski et al., 2007). In the present study, the bullfrogs were fed practical diets supplemented with synthetic amino acids and exhibited satisfactory mean values of growth and feed conversion for the environmental conditions, even when the diets were deficient in lysine. The growth results indicated that lysine is essential for bullfrogs, which were able to utilize L-lysine in practical diets.

Feed intake increased with increasing inclusion level of lysine in the diet until reaching its peak, estimated at 2.16% digestible lysine. The same pattern has been reported by Encarnação et al. (2004) for rainbow trout, by Furuya et al. (2006) and Bonfim et al. (2010) for Nile tilapia, and by Grisdale-Helland et al. (2011) for Atlantic cod. According to Furuya et al. (2004), feed intake is directly related to the increase in lysine levels.

According to Bureau and Encarnação (2006), an imbalance in dietary amino acids can cause a reduction in feed intake and in the efficiency of EAA utilization. This proposal was confirmed by Yamamoto et al. (2000) who tested the self-selection of diets by rainbow trout and observed that these animals prefer diets with an adequate amino acid balance.

The optimum level of digestible lysine (2.23%) estimated for the best weight gain of bullfrogs (222.7 g) was similar to the value reported by Encarnação et al. (2004) for rainbow trout (2.28%) using diets with 40% digestible protein.

Feed conversion improved with the inclusion of up to 2.29% digestible lysine in the diets, a value similar that observed for feed intake and live weight gain. These results indicate that the ideal level of digestible lysine in practical diets for bullfrogs can drastically change feed conversion, with a diet that is more concentrated in a certain nutrient, such as lysine, improving feed conversion (Sakomura and Rostagno, 2007). The best feed conversion estimated (1.40 g/g) was better than that reported by Castro et al. (2014a,b) of 2.11 and 2.13 g/g, respectively. These authors used commercial carnivorous fish feeds for bullfrogs, demonstrating the importance of an appropriately balanced amino acid ratio in the diet for these animals.

The highest specific growth rate (1.85% per day) estimated at 2.21% digestible lysine was higher than that observed by Martínez et al. (2004) for *Rana perezi* (1.08% per day) fed a diet containing 46% crude protein.

The deposition of protein and lysine showed the same trend, reaching maximum values at 2.33% and 2.39% digestible lysine, respectively. Furuya et al. (2006) also found greater protein deposition in the body composition of Nile tilapia with a linear increase in digestible lysine levels in the diet. The same fact was reported by Encarnação et al. (2004, 2006) and by El-Haroun and Bureau (2006) for lysine deposition in rainbow trout.

The best digestible lysine level (2.19%) estimated for the highest protein efficiency ratio (2.3 g/g) of bullfrogs was similar to the 2.17% crude lysine reported by Tibaldi and Lanari (1991) for sea bass (*Dicentrarchus zabrax* L.) and the 2.16% crude lysine estimated by Forster and Ogata (1998) for juvenile red sea bream (*Pagrus major*). However, in contrast to the present study, these authors used crude lysine. Olvera-Novoa et al. (2007) found a protein efficiency ratio of 1.93 g/g for bullfrogs fed 42% crude protein, a value lower than that observed in the present study (2.3 g/g).

The choice of protein retention efficiency to determine the digestible lysine requirements of bullfrogs (2.71%) is biologically more adequate compared to the other variables analyzed in this study, because amino acids are retained in protein and variables considering weight gain may be influenced by the gain provided by fat (Gaylord and Barrows, 2009).

The concept of the ideal amino acid ratio has been used to estimate the requirements of all EAA when one is known, based on the ideal ratio between one amino acid and all EAA in animal tissues (Arai, 1981; Kaushik, 1998; Kim and Lall, 2000). The estimate requirement

Table 4
Digestible essential amino acid (EAA) requirements of growing bullfrogs calculated based on the concept of the ideal ratio of digestible EAA to digestible dietary protein.

	% amino acids of body protein in growing bullfrogs[1]	EAA/ΣEAA ∗ 1000 (Arai, 1981)	Ideal EAA profile (%)[2]
Arginine	6.73	133.04	2.16
Histidine	2.94	58.21	0.94
Isoleucine	4.18	82.62	1.34
Leucine	7.45	147.33	2.39
Lysine	8.46	167.18	2.71
Methionine	2.47	48.78	0.79
Phenylalanine	4.09	80.82	1.31
Threonine	4.17	82.41	1.34
Tryptophan	0.70	13.92	0.23
Valine	4.93	97.39	1.58
Cystine	1.13	22.37	0.36
Tyrosine	3.33	65.93	1.07
ΣEAA + Cys + Tyr	50.58		

[1] Determined in a previous study.
[2] Estimated requirement of the other digestible EAA in relation to digestible lysine requirement: (2.71 / 167.18) × result of the formula of Arai (1981).

of digestible EAA using the concept of the ideal ratio permits the elaboration of diets with an appropriate amino acid balance (Fuller et al., 1989; Wang and Fuller, 1989; Boisen et al., 2000; Dari et al., 2005; Abimorad et al., 2010), maximizing growth, protein utilization efficiency and carcass quality of bullfrogs. The digestible lysine requirement of bullfrogs (2.71% of dry weight diet) was not so different from the requirements of carnivorous fish species (Arai, 1981; Kaushik, 1998; Kim and Lall, 2000).

5. Conclusion

The digestible lysine requirement of bullfrogs is 2.71% of dry weight or 8.91% of dietary digestible protein, a level that provides the highest protein retention efficiency. The requirements of the other digestible amino acids estimated based on the concept of the ideal ratio of EAA are (of dry weight): 2.16% arginine; 0.94% histidine; 1.34% isoleucine, 2.39% leucine; 0.79% methionine; 1.31% phenylalanine; 1.34% threonine; 0.23% tryptophan; 1.58% valine; 0.36% cystine, and 1.07% tyrosine.

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