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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).

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