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Apparent digestibility coefficients and amino acid availability of common protein ingredients in the diets of bullfrog, *Rana* (*Lithobates*) *catesbeiana*

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ABSTRACT

Farming of bullfrog *Rana* (*Lithobates*) *catesbeiana* has been expanding remarkably in the past decades to support an emerging food delicacy in southeastern China. However, lack of understanding of nutrient availability in common protein ingredients restricts precise formulation of bullfrog feeds, partially because of difficulties in collection of fecal material from bullfrog. Therefore, we carefully designed an apparatus that allows feces of bullfrogs to be collected while minimizing leaching in water and reducing stress imposed to the animals. By using this apparatus, a study was conducted to determine apparent digestibility coefficients (ADCs) of dry matter, crude protein, gross energy, and availability of phosphorus and amino acids in practical feed ingredients for bullfrog. Test ingredients included white fishmeal (WFM), brown fishmeal (BFM), porcine meat and bone meal (PMBM), poultry by-product meal (PBM), feather meal (FEM), soybean meal (SBM), peanut meal (PNM), cottonseed meal (CSM) and rapeseed meal (RSM). A reference diet and test diets that included 70% reference diet and 30% of the test feed ingredients were used with 0.5% chromic oxide included as a digestibility indicator. Bullfrogs (153 ± 3.14 g) were cultured in indoor aquaria ($68 \times 38 \times 38$ cm) equipped with the apparatus for fecal collection, and each aquarium was stocked with 15 specimens. ADCs of dry matter and energy were high (above 80%) in WFM and BFM, and low (below 40%) in CSM and RSM. ADCs of crude protein in those tested feed ingredients varied from 60.7 to 89.8%. WFM (89.8%), BFM (87.6%), SBM (83.3%), PNM (82.1%), PMBM (78.0%), PBM (77.6%) were more digestible protein feedstuffs, compared to RSM (69.5%), CSM (67.5%), and FEM (60.7%). In addition, the highest availability for phosphorus (84.8%) was observed in WFM, and the lowest value was found in FEM (63.6%). This study also demonstrated that phosphorus availability in the other seven ingredients ranged from 71.9 to 84.0%, indicating that phosphorus in BFM, PMBM, PBM, SBM, PNM, CSM and RSM could also be utilized by the bullfrog. Amino acid availability for the test ingredients followed a similar pattern to ADCs of crude protein, in which values for fishmeal were generally higher ($P < 0.05$) than those of other protein ingredients. Among all plant meals, the amino acid availability values in soybean meal were significantly ($P < 0.05$) higher than those in PNM, CSM and RSM. The availability of most amino acids in FEM was the lowest ($P < 0.05$) among all tested ingredients. In conclusion, PMBM, PBM, SBM and PNM may be good sources of available protein and amino acids, and should be promising substitutes for fishmeal in diets for bullfrogs.

1. Introduction

Bullfrog *Rana* (*Lithobates*) *catesbeiana* is one of the largest frogs of the genus *Rana*. It is an important economic amphibian native to North America that was introduced to China in the 1950s for aquacultural production. Bullfrog muscle in various cuisine styles has become food delicacies of international gastronomy that has increased worldwide consumption (Pasteris et al., 2006). The export of bullfrog legs to the western world has been increasing in the past years. Additionally, co-products of bullfrog meat processing including liver, gut and skin, also possess potential applications in pharmaceutical and biochemical industries (Pasteris et al., 2006). In recent years, bullfrog culture has been rapidly expanding in southeastern China, due to the animal's outstanding reproductive performance, fast growth rate, desirable culture environments and increased investment in infrastructure to produce these animals. In 2013, production of bullfrog in China was estimated to be 150,000 metric tons. However, the feed formulation technology for bullfrog culture is still in its very infancy. Bullfrog growers traditionally mix high concentration of fishmeal with ground offal of bullfrog, and feed that mixture. This feed practice results in considerable waste of fishmeal, excessive excretion of nitrogen and

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phosphorus, deteriorated water quality and increased risks of disease transmission due to environmental stress and cannibalism, as well as lack of destruction of resident microorganisms in the feed preparation process.

In the past decade, the dietary protein requirement (Carmona-Osalde et al., 1996; Olvera-Novoa et al., 2007) and optimal protein and lipid ratio (Huang et al., 2014) have been determined for the bullfrog or tadpoles by various researchers, and the optimal feeding frequency for the bullfrog was determined (Castro et al., 2012). Apparent digestibility of feed ingredients for the bullfrog tadpole also was estimated (Secco et al., 2005). However, the digestibility of various common protein ingredients for bullfrog has not been well studied.

The feeding behavior of the bullfrog has made fecal collection extremely difficult using either the conventional abdominal pressure (stripping) method, anal suction method or adapting the apparatus for collecting fish feces. Therefore, it is of special urgency to design an apparatus to collect feces of the bullfrog without leaching or imposing extra stress to study nutrient utilization of common protein ingredients.

The protein component of most aquafeeds is the single most important and expensive dietary component, especially for carnivorous or piscivorous species. Fishmeal is in high demand as a source of protein for many aquaculture feeds including bullfrog, because of its amino acid profile, availability, and other nutritional properties. In recent years, with rising fishmeal prices and reduced availability, replacement of fishmeal in aquafeeds has received heightened attention from academic institutions and industry.

The use of these alternative proteins in diets must be able to support similar animal performance and, concurrently, have little or no adverse effects upon animal health and the environment (Tibbetts et al., 2006). Common alternative protein ingredients include terrestrial animal protein meals produced by the modern rendering industry, plant protein meals and certain bacterial proteins. Selection of potential protein sources for feed formulation for any animals requires knowledge of digestibility of its nutrients. Consequently, the determination of digestibility is an essential tool not only to formulate diets that maximize growth of the animal but also to reduce waste production (Secco et al., 2005) because precisely formulating diets based on nutrient requirements and available nutrients in the ingredients may allow nitrogen and phosphorus excretion to be significantly reduced (Primavera, 2005). As such, the purpose of this study was to determine apparent digestibility of dry matter, protein, gross energy, phosphorus and amino acids in common protein ingredients including white fishmeal (WFM), brown fishmeal (BFM), porcine meat and bone meal (PMBM), poultry by-product meal (PBM), feather meal (FEM), soybean meal (SBM), peanut meal (PNM), cottonseed meal (CSM) and rapeseed meal (RSM) for bullfrog.

2. Materials and methods

2.1. Design of apparatus for fecal collection

We tried to adapt the conventional abdominal pressure (stripping) method, anal suction method as well as adapt the apparatus for collecting feces from fish in preliminary trials. Both abdominal pressure and anal suction imposed severe stress on the bullfrog. Anesthetics such as MS-222 designed for fish failed to anesthetize the bullfrog, while the use of carbon dioxide was not pursued because it may have caused undetermined effects. Therefore, we designed an apparatus to be fitted into experimental aquaria that allowed the bullfrog's feces to be collected under normal culture conditions (Fig. 1A). This apparatus consisted of five components: a frame, a screen, a sieve, support blocks and feces collecting chamber (Fig. 1B). The screen was made of plastic with 1.5-cm pore size. It was fixed on the top of the frame to support the weight of bullfrogs, so the droppings could fall through the screen. The consistency of the bullfrog's feces is a viscous solid. Three rectangular 5-cm-high support blocks were mounted on the bottom of the frame. The space between the screen and support blocks was the chamber for fecal collection. A movable sieve plate was placed at the bottom of the feces collecting chamber, as shown in Fig. 1B. This sieve was covered with filter paper to instantly remove splashed water and urine on the surface of the droppings. After feeding the bullfrogs at 08:30, the apparatus was placed on the bottom of each aquarium with 3 cm of water covering the bottom. This apparatus occupied 60% of the bottom of each aquarium (Fig. 1A), which allowed bullfrog to move freely, rest and defecate without apparent stress. After 23 h, the apparatus was removed from the aquarium, and the sieve plate was pulled out, and feces were collected.

2.2. Diet formulation and preparation

The reference diet was formulated to contain about 39% crude protein and 4.0% crude lipid by using fishmeal and soybean meal as the main protein sources, fish oil as the main lipid source (Table 1). Wheat flour was included in the formulation for pelleting purposes. Vitamin and mineral premixes were included to supply micro-nutrients to the bullfrog. Apparent digestibility coefficients were determined by using the reference diet and test diets. The reference diet contained 99.5% of the basal mixture and 0.5% chromic oxide. The test diets were produced containing 69.65% of the basal mixture and 29.85% of the test feed ingredient. All experimental diets also were supplemented with 0.5% chromic oxide as an inert indicator. Apparent digestibility coefficients for dry matter, protein, energy and amino acid availability were determined for nine feed ingredients including white fishmeal (WFM), brown fishmeal (BFM), porcine meat and bone meal (PMBM), poultry by-product meal (PBM), feather meal (FEM), soybean meal (SBM), peanut meal (PNM), cottonseed meal (CSM) and rapeseed (RSM) meal. The proximate composition and amino acid profiles of each test ingredient are summarized in Table 2.

Prior to preparing the diets, the feed ingredients were ground and sieved, so the particles could pass through a 250-μm sieve. The dry ingredients of each diet were mixed thoroughly in a mixer before lecithin and fish oil were added. After the oil was dispersed, water was added and mixed. Then the mixture was conveyed into a MY45 single screw extrusion machine (Xiamen fishing machinery feed machinery Co., Ltd.) with a 2.5-mm die to produce neutrally buoyant 4.5 × 6.5-mm pellets. The pellets were dried to a moisture content of about 10% in a forced-air environment at 20 °C for about 20 h, and then stored at −20 °C until used.

2.3. Animals and experimental procedures

Bullfrogs were obtained from a commercial farm and fed a commercial feed (FROGS FORMULA FEED-2#) from Xiamen Baisuihang Technology Co., Ltd (Xiamen, Fujian, PR China), which was about 4.8-mm in diameter and contained 38.6% crude protein, and 4.3% crude lipid. Prior to the start of the experiment, bullfrogs were reared in indoor aquarium (150 × 70 × 58 cm) and fed twice daily (08:00 and 16:00) with the reference diet for 15 days to acclimate to the experimental conditions. At the start of the experiment, bullfrogs (153 ± 3.14 g initial body weight) were randomly sorted into 30 aquaria (68 × 38 × 38 cm), and each aquarium was stocked with 15 bullfrogs. Each dietary treatment was randomly assigned to three aquaria, and each diet was fed to bullfrogs in the chosen aquaria by hand to visual satiation twice daily at 08:00 and 17:00 h. The fecal samples from each replicate aquarium were individually collected, quickly frozen and stored at −20 °C until analyzed. During the experimental period, the water temperature was maintained at 25 ± 2 °C. Fecal samples from each aquarium were collected for a total of 30 days.