Lat. Am. J. Aquat. Res., 43(4): 786-791, 2015
DOI: 10.3856/vol43-issue4-fulltext-18

Short Communication

Apparent digestibility of energetic ingredients by pirarucu juveniles, *Arapaima gigas* (Schinz, 1822)

Filipe dos Santos-Cipriano[1], Kauana Santos de Lima[2], Érica Bevitório-Passinato[2]
Raildo Mota de Jesus[2], Francisco Oliveira de Magalhães Júnior[2],
William Cristiane Teles-Tonini[6] & Luis Gustavo Tavares-Braga[7]
[1]Universidade Federal de Minas Gerais, Belo Horizonte, MG, CEP 31270901, Brasil
[2]Universidade Estadual de Santa Cruz, Ilhéus, BA, CEP 45662900, Brasil
[3]Universidade do Estado da Bahia, Xique-Xique, BA, CEP 47400000, Brasil
Corresponding author: Gustavo Braga (lgtbraga@gmail.com)

ABSTRACT. An understanding of feed ingredient digestibility for the pirarucu is a fundamental step in the development of feeds that promote proper growth of the specie while in captivity. A digestibility trial was conducted with four treatments in triplicate (corn starch, corn, rice bran and wheat bran) to evaluate the digestibility of dry matter, gross energy, crude protein and amino acids by the pirarucu. We used indirect methodology with the inclusion of chromium oxide at 0.1% in the feeds. In total, 18 juveniles were used, with an average live weight of 235 ± 36 g. The sampled juveniles were trained to consume the feeds prior to testing. The corn and cornstarch presented the best apparent digestibility coefficients (ADCs) of dry matter, with 76.37% and 70.66%, respectively, followed by rice bran (46.23%) and wheat bran (45.13%). The best ADCs of crude protein were observed in corn (93.44%) and cornstarch (90.94%) compared to rice bran (68.23%) and wheat bran (68.58%). There was no significant difference in the ADC of gross energy; the values ranged from 47.10% for corn starch to 40.10% for corn. The corn and corn starch presented the best ADCs for all the amino acids evaluated, followed by rice bran and wheat bran.

Keywords: *Arapaima gigas*, carnivorous, feeding, nutritional value, protein, aquaculture.

Digestibilidad aparente de ingredientes energéticos por juveniles de pirarucu, *Arapaima gigas* (Schinz, 1822)

RESUMEN. El conocimiento de la digestibilidad de los ingredientes en la alimentación del pirarucu es primordial para la elaboración de pienso específico, que promueva un crecimiento óptimo. Se realizó un ensayo de digestibilidad con cuatro ingredientes energéticos, almidón de maíz, harina de maíz, salvado de arroz y salvado de trigo para la evaluación de las digestibilidades de materia seca, energía bruta, proteína bruta y aminoácidos. Se utilizaron 18 juveniles con peso de 235 ± 36 g. La harina de maíz y el almidón de maíz presentan los mejores coeficientes de digestibilidad aparente (CDA) de la materia seca, 76,37% y 70,66% respectivamente, seguidos por el salvado de arroz (46,23%) y salvado de trigo (45,13%). Los mejores CDA de la proteína bruta se determinaron para harina de maíz (93,44%) y almidón de maíz (90,94%), en relación al salvado de arroz (68,23%) y salvado de trigo (68,58%). Para el CDA de la energía bruta no fue registrada diferencia estadística, variando entre 47,10% para el almidón de maíz y a 40,10%, para la harina de maíz. La harina de maíz y el almidón de maíz presentaron los mejores CDA de todos los aminoácidos evaluados, seguidos por el salvado de arroz y salvado de trigo.

Palabras clave: *Arapaima gigas*, valor nutritivo, alimentación, carnívoro, proteína, acuicultura.

The pirarucu, *Arapaima gigas*, is a carnivorous fish endemic to the Amazon basin. The fish offers great potential for use in aquaculture; its meat is highly utilized, it provides a good carcass yield, and it has rapid growth, with the capacity to reach more than 10 kg in one year of cultivation (Imbiriba, 2001).

Knowledge of the digestibility of the ingredients used in fish feed is of fundamental importance because it allows the formulation of more efficient feeds, thus resulting in a better utilization of nutrients, an optimization of feeding costs and an increase in productivity and profitability for the producer. Our ob-

Corresponding editor: Jesús Ponce

jective was to determine the apparent digestibility coefficient of the dry matter, crude protein, gross energy and amino acids of the energetic ingredients by pirarucu juveniles.

The experiment was conducted at the laboratory of fish nutrition and feeding (AQUANUT) at the State University of Santa Cruz in October 2012; the experimental period was 18 days. We used 18 juvenile pirarucu, with an average live weight of 235 ± 36 g; specimens were provided by the Canta Galo Farm, Ibirataia-Ba. Three individuals were housed per tank in six tanks (310 L) that were later used as feeding tanks. The tanks were arranged in a closed circulation system using a water pump (Dancor ®, RJ, Brazil-75 HP) with biological filters, and constant aeration was provided by a blower (WEG of 1 HP).

Juveniles were subjected to period of adaptation to laboratory and routine management conditions for ten days, during which they received the reference feed (Table 1) four times a day. During the adjustment period and the experimental period, daily cleaning was performed to remove feces and possible scraps of feed.

The reference ration was formulated using the SUPER CRAC® computational program, which monitored the crude protein levels as tested by Ituassú *et al*. (2005). In total, 1 g kg^{-1} of chromium oxide (Cr_2O_3) was added as an external indicator of the feeds for the determination of digestibility. For the manufacture of feed, the ingredients were ground in a knife type mill, passed through a 0.5 mm sieve and then homogenized in accordance with the formulation of each feed. The feeds were processed in a meat grinder with a reverser using a matrix of 2 mm. Prior to processing, water (40°C) was added to the mixture. Feed grains were subsequently dried in a forced ventilation oven (55°C) for 24 h and disintegrated to an appropriate size for fish consumption. The test feeds were formulated using a mixture of 70% of the reference feed with 30% of the ingredients to be tested (Table 2). We evaluated the apparent digestibility coefficients of four energetic ingredients: corn, corn starch, wheat bran, and rice bran. For each ingredient, we used three replications.

For each treatment, the fish remained in the tanks (310 L) during the daytime period where they received five feedings per day, two in the morning (08:00 and 10:00 h) and three in the afternoon (12:00, 14:00 and 16:00 h). An hour after the last feeding, the fish were transferred to the digestibility aquariums (200 L) to perform feces collection. The digestibility aquariums had a conical shape with a constant aeration system and were equipped with collectors at the bottom that were submerged in water and ice during the collection periods. At 07:00 h the next day, the fish were transferred to the supply tanks; next, the collectors were removed and the material was collected. For each feed, the fish were subjected to a three-day adaptation period and a subsequent three-day period of feces collection.

The apparent digestibility coefficients of the feed and the test ingredients were verified using indirect methods with the use of chromium oxide as the external indicator. The apparent digestibility coefficients of the rations (ADC_{RA}) were calculated according to De Silva (1989). The coefficients of digestibility of the ingredients (ADC_I) were calculated using the methodology employed by Bureau *et al*. (1999).

After the withdrawal of the collectors containing the water and feces, the collected material was released from the water contained in the upper third of the collector and added to the remaining volume in disposable aluminum containers for drying in a forced ventilation oven at 55ºC for 12 h. After drying and checking for the possible presence of scales, the samples were identified, stored in plastic containers and kept in a freezer (-10°C) for subsequent laboratory analysis of dry matter (DM), mineral matter (MM), crude protein (CP), gross energy (GE) and the concentration of chromium. The analysis of crude protein, crude energy, dry matter and mineral matter were performed in the laboratory of animal nutrition and in the laboratory of fish nutrition and feeding at the State University of Santa Cruz, according to AOAC (2005) methodology. The analyses of amino acids of the feeds and feces were performed using ionic chromatography (Evonik Industries AG). Chromium concentrations were analyzed at the Electron Microscopy Centre at the State University of Santa Cruz in an optical emission spectrometer with inductively coupled plasma (ICPO-ES), Varian model 710-S series.

The physicochemical variables of the water, pH, temperature (ºC) and dissolved oxygen (mg L^{-1}) were monitored daily throughout the trial period using YSI Professional Plus multi-parameter equipment and presented the values of 6.8-7.0, 26.8 ± 0.43°C and 7.2 ± 1.43 mg L^{-1}, respectively.

Data were subjected to variance analysis and the differences between the averages were submitted to the Scott-Knott test at 5% probability using the statistical program R Core Team (2011).

The apparent digestibility coefficients (ADC) of dry matter, crude protein and gross energy of the ingredients evaluated for juvenile pirarucus showed significant differences (Table 3). The highest apparent digestibility coefficients of dry matter (ADCDM) were found for corn and cornstarch. The rice bran and wheat did not differ between each other, with both shows in lower ADCDM.

Table 1. Diets composition for pirarucu juveniles.

Ingredients (g kg^{-1})	Diet				
	Reference	Corn starch	Rice bran	Corn	Wheat bran
Soybean meal	188.00	130.80	130.80	130.80	130.80
Wheat bran	140.00	97.41	97.41	97.41	397.41
Corn gluten meal	105.00	73.06	73.06	73.06	73.06
Corn	90.03	62.64	62.64	362.64	62.64
Fish meal	370.00	257.44	257.44	257.44	257.44
Rice bran	-	-	300	-	-
Poultry by-product meal	57.67	40.13	40.13	40.13	40.13
Corn starch	27.00	318.79	18.79	18.79	18.79
Soybean oil	8.45	5.88	5.88	5.88	5.88
Mineral and vitamin mix[1]	7.00	7.00	7.00	7.00	7.00
Sodium chloride	3.50	3.50	3.50	3.50	3.50
Cellulose	2.15	2.15	2.15	2.15	2.15
Chromic oxide III	1.00	1.00	1.00	1.00	1.00
BHT[2]	0.20	0.20	0.20	0.20	0.20
Total	1000	1000	1000	1000	1000
Crude protein	432.40	301.81	339.87	316.15	342.15
Gross energy (kJ g^{-1})	19.63	18.67	20,39	20.29	19.68
Ash	143.00	123.68	137.94	117.42	123.68

[1]Mineral and vitamin mix per kg of product: vitamin A 6000000 UI, vitamin D3 2250000 UI, vitamin E 75000 mg, vitamin K3 3000 mg, vitamin tiamine (B1) 5000 mg, riboflavin (B2) 10000 mg, pirodoxine 8000 mg, biotin 2000 mg, ascorbic acid (vitamin C) 192500 mg, niacin 30000 mg, folic acid 3000 mg, Fe 100000 mg, Cu 600 mg; Mn 60000 mg, Zn 150000 mg, I 4500 mg, Cu 15000 mg, Co 2000 mg, Se 400 mg[2] Butyl-hydroxy-toluene.

Similarly, it was observed for the apparent digestibility coefficient of crude protein (ADCCP), in which the ingredients that demonstrated the highest digestibility were corn and cornstarch; the digestibility did not differ between corn and cornstarch, while rice bran and wheat bran, showed digestibility below 70%. The ADC of all amino acids was higher for cornstarch and corn, the minor values were found for the rice bran and wheat bran.

There was no significant difference for the apparent digestibility coefficient of energy (ADCGE) among all the ingredients. All the ingredients presented low digestibility; the values ranged between 40.10 and 47.87%.

The best ADCDM were found for corn and cornstarch. Rice bran and wheat bran showed lower ADCDM, most likely due to the high ash content (Table 2) contained in the two ingredients and the higher levels of phytate (Kumar *et al*., 2012), which partially reduces the availability of minerals, in addition to the higher fiber content present in wheat bran.

Another factor that likely contributed to the lower rice bran and wheat bran ADCDM is the large amount of non-starch polysaccharides in these two ingredients. According to Conte *et al*. (2003), this soluble fiber has the ability to absorb water, making the digested material more viscous and reducing the activity of enzymes and nutrient absorption. This result was also found by Glencross *et al.* (2012a), who studied the digestibility of different sources of starch and non-starch polysaccharides in trout (*Oncorhynchus mykiss*). Teixeira *et al*. (2010) studied the digestibility of energetic ingredients in *Pseudoplatystoma* sp. and found a lower ADCDM for corn (62.30%) and a higher ADCDM for rice bran (59.67%) than the results of this study.

In previous studies of carnivorous freshwater fish, several researchers found lower values for the protein digestibility of corn, with 51.4% for surubim *Pseudoplatystoma reticulatum* (Silva *et al*., 2013), and 64.18% for painted *Pseudoplatystoma corruscans* (Gonçalves & Carneiro, 2003). For the carnivorous marine fish "red drum", *Sciaenops oceallatus*, the ADCCP found by Mc Googan & Reigh (1996) was lower for corn (81.56%) and slightly higher for rice bran (77.16%). Wheat bran and rice bran showed the lowest ADCCP. These results were similar to those of Gonçalves & Carneiro (2003). The authors studied the digestibility of ingredients in the painted *Pseudoplatystoma corruscans*, in which they observed higher digestibility with corn compared to wheat bran and rice bran.

Table 2. Chemical composition of ingredients for pirarucu juveniles.

	Ingredients			
	Corn starch	Rice bran	Corn	Wheat bran
Dry matter (g kg^{-1})	88.62 ± 0.2	91.72 ± 0.4	88.62 ± 0.3	88.56 ± 0.4
Crude protein (g kg^{-1})	14.35 ± 2.0	138.06 ± 5.3	80.75 ± 7.2	177.8 ± 8.2
Gross energy (kJ g^{-1})	17.09 ± 0.6	21.34 ± 0.5	16.88 ± 0.4	18.05 ± 0.8
Lipid (g kg^{-1})	8.71 ± 0.8	217.90 ± 6.6	85.56 ± 1.3	116.11 ± 3.0
Ash (g kg^{-1})	1.60 ± 0.3	100.34 ± 1.1	8.65 ± 0.4	50.36 ± 0.6
Amino acids (%)				
Ala	0.159	0.742	0.532	0.792
Arg	0.096	0.822	0.316	1.153
Asp	0.210	1.060	0.471	1.143
Cys	0.019	0.229	0.159	0.286
Glu	0.380	1.500	1.370	2.920
Gly	0.136	0.726	0.276	0.900
His	0.061	0.274	0.244	0.367
Ile	0.073	0.432	0.306	0.516
Leu	0.213	0.809	0.903	0.993
Lys	0.159	0.292	0.085	0.625
Met	0.062	0.308	0.175	0.265
Phe	0.088	0.502	0.355	0.615
Pro	0.099	0.572	0.619	0.886
Ser	0.141	0.515	0.271	0.682
Thr	0.101	0.448	0.237	0.521
Val	0.076	0.629	0.396	0.726

Table 3. Apparent digestibility coefficient (ADC) of: dry matter, crude protein and energy, and amino acid of tested ingredients for pirarucu juveniles. Values followed by the same superscripts within columns do not differ ($P > 0.05$).

	Ingredients (%)				CV (%)	(*P*)
	Corn starch	Rice bran	Corn	Wheat bran		
Dry matter	70.66^{a} ± 2.54	46.23^{b} ± 2.79	76.37^{a} ± 0.42	45.13^{b} ± 0,80	5.98	0,0010
Crude protein	90.94^{a} ± 3.50	68.23^{b} ± 6.27	93.44^{a} ± 3.44	68.58^{b} ± 2,25	6.39	0,0020
Gross energy	47.87^{a} ± 5.37	42.23^{a} ± 2.35	40.10^{a} ± 5.42	47.37^{a} ± 3.67	9.90	0,1560
Amino acids						
Ala	89.41^{a}	55.64^{b}	91.95^{a}	57.35^{b}	9.71	0.0003
Arg	97.38^{a}	77.20^{b}	96.67^{a}	72.29^{b}	5.95	0.0005
Asp	91.76^{a}	55.90^{b}	89.36^{a}	48.50^{b}	13.84	0.0011
Cys	84.32^{a}	41.86^{b}	83.10^{a}	50.17^{b}	15.32	0.0013
Glu	95.16^{a}	69.80^{b}	96.34^{a}	70.38^{b}	7.60	0.0008
Gly	94.84^{a}	70.01^{b}	90.65^{a}	67.48^{b}	6.87	0.0005
His	93.64^{a}	68.62^{b}	92.18^{a}	66.88^{b}	7.92	0.0010
Ile	89.08^{a}	51.95^{b}	89.71^{a}	49.02^{b}	14.68	0.0014
Leu	94.41^{a}	61.26^{b}	94.41^{a}	59.41^{b}	10.59	0.0007
Lys	94.42^{a}	60.33^{b}	93.42^{a}	60.98^{b}	10.07	0.0006
Met	91.24^{a}	60.92^{b}	89.58^{a}	56.00^{b}	9.15	0.0003
Phe	84.27^{a}	39.81^{b}	80.80^{a}	52.76^{b}	15.74	0.0016
Pro	92.90^{a}	66.80^{b}	91.12^{a}	72.64^{b}	6.21	0.0003
Ser	92.56^{a}	58.80^{b}	89.11^{a}	58.21^{b}	10.22	0.0006
Thr	87.22^{a}	44.80^{b}	80.72^{a}	42.50^{b}	13.96	0.0004
Val	90.28^{a}	54.25^{b}	88.54^{a}	51.29^{b}	12.61	0.0008

The diets formulation based on the amount of available amino acids can result in significant improvements in performance (Rawles *et al*., 2006). The digestibility of amino acids tend to reflect protein digestibility, however differences may occur in the ADCAA of some amino acids (Zhang *et al*., 2015). In this study, similar to ADCCP, corn and corn starch showed the highest values for the ADC of all amino acids. Among the amino acids, lysine is considered the first limiting to the growth of fish (Abboudi *et al*., 2006). Methionine is required in large quantities and also plays an important role in growth (Bomfim *et al*., 2008). The values of the ADC of lysine and methionine found by Ribeiro *et al*. (2011) using Nile tilapia are slightly lower than the present work for corn (80.38 and 80.87%) and slightly higher for wheat bran (79.92 and 79.66%, respectively).

All ingredients showed an ADCGE below 50%. The test ingredients had high levels of starch; typically, carnivorous fish species present less activity of amylase compared to omnivorous species (Hidalgo *et al*., 1999). Glencross *et al*. (2012b) observed a negative relationship between the higher levels of amylopectin and digestibility in juveniles of *Lates calcarifer*. Similar to the observations of this study, Silva *et al*. (2013) and Lundstedt *et al*. (2004) observed difficulties in the use of starch as an energy source by the carnivorous fish *P. reticulatum* and *P. corruscans*, respectively.

Lower ADCGE levels for ingredients of vegetable origin compared to ingredients of animal origin were observed for the carnivorous fish *Rachycentron canadum* (Zhou *et al*., 2004), *Sebastes schlegeli* (Lee, 2002) and *P. corruscans* (Gonçalves & Carneiro, 2003). Low ADCGE values were also found by Gonçalves & Carneiro (2003) for wheat bran (53.20%), rice bran (47.34%), and corn (64.95%) using *P. corruscans* (9.80 g). Silva *et al*. (2013) observed low ADCGE values for corn (43.24%) and wheat bran (40.45%) in *P. reticulatum* (82.40 g). Braga *et al*. (2008) evaluated the digestibility of ingredients in *Salminus brasiliensis* juveniles (35.51 g) and found that the ADCGE values were the highest for corn (80.84%) and wheat bran (77.02%).

In conclusion, pirarucu exhibited a good ability to utilize protein from corn and cornstarch, although the species was not able to efficiently digest the energy contained in any of the tested ingredients.

ACKNOWLEDGMENTS

This project was supported by Coordination for the Improvement of Higher Education Personnel (CAPES) and Bahia Research Foundation (FAPESB-TSC0015/ 2012), Brazil. The Cantagalo Farm (Bahia, Brazil) provided the fish for this project.

REFERENCES

Abboudi, T., M. Mambrini, W. Ooghe, Y. Larondelle & X. Rollin. 2006. Protein and lysine requirements for maintenance and for tissue accretion in Atlantic salmon (*Salmo salar*) fry. Aquaculture, 261: 369-383.

Association of Official Analytical Chemists (AOAC). 2005. Official methods of analysis of AOAC, Maryland, 1234 pp.

Bomfim, M.A.D., E.A.T. Lanna, J.L. Donzele, A.S. Ferreira, F.B. Ribeiro & S.S. Takishita. 2008. Methionine plus cystine requirement based on ideal protein concept, in diets for Nile tilapia fingerlings. Rev. Bras. Zootec., 37: 783-790.

Braga, L.G.T., R. Borghesi & J.E.P. Cyrino. 2008. Apparent digestibility of ingredients in diets for *Salminus brasiliensis*. Pesqui. Agropec. Bras., 43: 271-274.

Bureau, D.P., A.M. Harris & C.Y. Cho. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 180: 345-358.

Conte, A.J., A.S. Teixeira, E.T. Fialho, N.A. Schoulten & A.G. Bertechini. 2003. Effect of phytase and xilanase on the performance and bone characteristics of broiler chicks fed diets with rice bran. Rev. Bras. Zootec., 32: 1147-1156.

De Silva, S.S. 1989. Digestibility evaluations of natural and artificial diets. Asian Fish Soc. Spec., 4: 36-45.

Glencross, B., N. Rutherford & N. Bourne. 2012a. The influence of various starch and non-starch polysaccharides on the digestibility of diets fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 356: 141-146.

Glencross, B., D. Blyth, S. Tabrett, N. Bourne, S. Irvin, M. Anderson, T. Fox-Smith & R. Smullen. 2012b. An assessment of cereal grains and other starch sources in diets for barramundi (*Latescal carifer*) implications for nutritional and functional qualities of extruded feeds. Aquacult. Nutr., 18: 388-399.

Gonçalves, E.G. & D.J. Carneiro. 2003. Apparent digestibility coefficients of protein and energy of some ingredients used in diets for pintado, *Pseudoplatystoma corruscans* (Agassiz, 1829). Rev. Bras. Zootec., 32: 779-786.

Hidalgo, M.C., E. Urea & A. Sanz. 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquaculture, 170: 267-283.

Imbiriba, E.P. 2001. Production potential of pirarucu, *Arapaima gigas*, in captivity. Acta Amaz., 31: 299-316.

Ituassú, D.R., M. Pereira-Filho, R. Roubach, R. Crescêncio, B.A.S. Cavero & A.L. Gandra. 2005. Crude protein levels for juvenile pirarucu. Pesqui. Agropec. Bras., 40: 255-259.

Kumar, V., A.K. Sinha, H.P.S. Makkar, G. Boeck & K. Becker. 2012. Phytate and phytase in fish nutrition. J. Anim. Physiol. Anim. Nutr., 96(3): 335-364.

Lee, S.M. 2002. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). Aquaculture, 207: 79-95.

Lundstedt, L.M., J.F.B. Melo & G. Moraes. 2004. Digestive enzymes and metabolic profile of *Pseudoplatystoma coruscans* (Teleostei: Siluriformes) in response to diet composition. Biochem. Mol. Biol., 137: 331-339.

McGoogan, B.B. & R.C. Reigh. 1996. Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets. Aquaculture, 141: 233-244.

Rawles, S.D., M. Riche, T.G. Gaylord, J. Webb, D.W. Freeman & M. Davis. 2006. Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (*Morone chrysops* ♀ × *M. saxatilis* ♂) in recirculated tank production. Aquaculture, 259: 377-389.

Received: 5 January 2015; Accepted: 16 June 2015

Ribeiro, F.B., E.A.T. Lanna, M.A.D. Bomfim, J.L. Donzele, M. Quadros & P.S.L. Cunha. 2011. True and apparent digestibility of protein and amino acids of feed in Nile tilapia. Rev. Bras. Zootec., 40: 939-946.

Silva, T.S.C., G.V. Moro, T.B.A. Silva, J.K. Dairiki & J.E.P. Cyrino. 2013. Digestibility of feed ingredients for the striped surubim *Pseudoplatystoma reticulatum*. Aquacult. Nutr., 19: 491-498.

Teixeira, E.A., E.O.S. Saliba, A.C.C. Euler, P.M.C. Faria, D.V. Crepaldi & L.P. Ribeiro. 2010. Apparent digestibility coefficients of different energetic ingredients for surubim juveniles. Rev. Bras. Zootec., 39: 1180-1185.

Zhang, C.X., K.K. Huang, L. Wang, K. Song, L. Zhang & P. Li. 2015. Apparent digestibility coefficients and amino acid availability of common protein ingredients in the diets of bullfrog, *Rana* (*Lithobates*) *catesbeiana*. Aquaculture, 437: 38-45.

Zhou, Q.C., B.P. Tan, K.S. Mai & Y.J. Liu. 2004. Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum*. Aquaculture, 241: 441-451.