

Research Article

Contributions to the nutrition of the American crocodile *Crocodylus acutus* (Cuvier, 1807) in captivity

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ABSTRACT. In this work we evaluated the essential amino acid profile of muscle *Crocodylus acutus* and calculated their chemical score of main proteins used in their diet in captivity. The separation and identification of amino acids was carried out by high performance liquid chromatography coupled to a fluorescence detector. The calculation of chemical score was obtained by dividing the value of each essential amino acid between the same amino acid of the reference protein. The lowest value of the relationships is the chemical score. The amino acids present in greater quantities in the tail muscle are glutamic acid, lysine and leucine (16.96, 9.84 and 8.87 g amino acid per 100 g of protein, respectively), whereas histidine, methionine and tryptophan (2.99, 2.93 and 0.59 g amino acid per 100 g of protein) were the lowest. The chemical score obtained results showed that the proteins of animal origin, preferably marine fish and supplemented with terrestrial animals ingredients, including beef liver appear to be the most effective. The incorporation of plant proteins in diets for *C. acutus* does not appear as a viable alternative due to deficiencies in several essential amino acids such as methionine, lysine and threonine.

Keywords: crocodile, diet, feeding, protein, amino acids, chemical score.

INTRODUCTION

Most nutritional studies of crocodiles in the wild are based on the analysis of stomach content and behavioral observations (Villegas & Schmitter-Soto, 2008). These reptiles are generalist predators that feed on a wide variety of prey, among which include: fish, arthropods, snails, crustaceans, turtles, and mammals (Thorbjarnarson *et al.*, 2006; Villegas & Schmitter-Soto, 2008; Cupul-Magaña *et al.*, 2008, 2010; Hernández-Hurtado *et al.*, 2011a, 2011b; Laverty & Dobson, 2013). In the case of crocodiles kept in captivity, studies are based mostly on the analysis of different formulated feeds and mixtures with different proportions of them and their effect on growth and sur-

vival (Hernández-Hurtado, 2008; Pérez-Gómez *et al.*, 2009; Hernández-Hurtado *et al.*, 2012).

Additionally, there are studies that have provided basic and essential nutritional information of crocodiles through their physiology and biochemistry (Herbert & Coulson, 1975; Barboza *et al.*, 2011), they have been scattered and fragmented, and none are related to *Crocodylus acutus* (Cuvier, 1807). It is necessary to study feeding and nutritional requirements for this species in captivity on a biochemical and physiological basis, avoiding protocols with trial and error to design diets that meet optimal nutritional requirements to allow proper development.

The present study deals with basic aspects of nutrition of *C. acutus* to increase knowledge about ma-

nagement of this species in captivity and its most efficient diet.

MATERIALS AND METHODS

Specimens

Six month old juvenile crocodiles *C. acutus* were obtained from the Unidad de Manejo para la Conservación de la Vida Silvestre (UMA) Reptilario Cipactli with coding to the Secretaría de Medio Ambiente y Recursos Naturales: INE/CITES/DGVS-CR-IN-0610-JAL/00 located in Puerto Vallarta, Jalisco, Mexico. Six, originally healthy crocodiles were obtained after euthanization by hypothermia, during the winter 2010. Less than one hour after death, they were frozen at -20°C until further analysis.

Amino acid profile

Samples of the caudal appendage frozen muscle were transported at -20°C to the Proximate Analysis and Laboratory of Comparative Physiology and Functional Genomics Laboratory at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) for lyophilization and defatting. Thereafter, freeze-dried tissues were then sent to the Laboratory of Nutrition at the Centro de Investigación en Alimentación y Desarrollo (CIAD) for amino acid (AA) composition analysis, as described by Vázquez-Ortiz *et al.* (1995).

Chemical score (CS)

Calculations of chemical scores were performed according to Block & Mitchel (1946) using the ratio of g of the essential amino acid (EAA) in the food protein to the same amount of the corresponding AA in the reference protein. The scoring pattern was the AA composition of crocodile muscle. CS = content of the EAA in the test protein/content of the EAA in the reference protein. The AA with the lowest score is the limiting amino acid (LAA), and the ideal relationship would have a value of 1, or very close to 1.

Proteins that are commonly used in the nutrition of this species, or could be considered as partial substitutes for animal-based feeds, were determined from published data of the US Department of Agriculture food composition tables (USDA, 2017) as well as other published research, in which the sample processing and hydrolysis method for AA analysis coincide with the performed procedure used in this study. The list included beef liver, tuna muscle (*Euthynnus pelamis* Linnaeus, 1758), channel catfish muscle (*Ictalurus punctatus*, Rafinesque, 1818), soybean, whole wheat, and yellow maize meals (USDA, 2017), muscle from Nile crocodile

(*Crocodylus niloticus* Laurenti, 1768) (Hoffman *et al.*, 2000), chicken and piglet carcasses, and whole tilapia (*Oreochromis niloticus* Linnaeus, 1758) (Pinheiro & Lavorenti, 2001).

RESULTS

Table 1, shows the amino acid profile of the tail muscle of *C. acutus*. The amino acids present in greater quantities in the tail muscle are glutamic acid, lysine and leucine (16.96, 9.84 and 8.87 amino acid per 100 g of protein, respectively), whereas histidine, methionine and tryptophan (2.99, 2.93 and 0.59 g amino acid per 100 g of protein) were the lowest.

Table 2, shows the profile of EAA of *C. acutus* muscle and the CS of protein from terrestrial animal protein sources that have been reported for feeding crocodiles (bovine liver, chicken muscle, chicken and piglet carcasses). It also shows the EAA profile of the Nile crocodile *C. niloticus*. Beef liver has identical chemical scores as *C. acutus* muscle, particularly lysine and threonine. For chicken meat, chicken carcasses, and pig carcasses, lysine was the first LAA. The second and third LAA varies from one protein source to another.

Table 3 shows the chemical score of aquatic animal proteins. In tilapia and catfish, histidine is first LAA. In tilapia, lysine, leucine, isoleucine, and threonine have very close scores, indicating that, if that source is supplied as a single feed, it would only cover 75-80% of the AA requirements for *C. acutus*. The same applies

Table 1. Amino acid profile of the muscle protein of *Crocodylus acutus*, AA: amino acids.

Amino acid	g amino acid per 100 g of protein (mean \pm SD)
Aspartic acid	8.43 \pm 0.29
Glutamic acid	16.96 \pm 1.34
Serine	3.31 \pm 0.17
Histidine	2.99 \pm 0.09
Glycine	5.64 \pm 0.49
Threonine	5.33 \pm 0.34
Alanine	6.59 \pm 0.41
Arginine	5.88 \pm 0.13
Tyrosine	5.44 \pm 0.16
Valine	4.82 \pm 0.26
Phenylalanine	4.45 \pm 0.12
Isoleucine	5.24 \pm 0.09
Leucine	8.87 \pm 0.87
Lysine	9.84 \pm 0.33
Methionine	2.93 \pm 0.07
Tryptophan	0.59 \pm 0.004
Muscle protein content	67%

Table 2. Essential amino acid profile (EAA) of *Crocodylus acutus* muscle, and chemical score (CS) of terrestrial animals protein muscle, used or proposed in its diet (g of amino acids per 100 g of protein). *Limiting amino acids 1) Centro de Investigación en Alimentación y Desarrollo (CIAD) (México), 2) USDA (2017), 3) Pinheiro & Lavorenti (2001), 4) Hoffman *et al.* (2000).

Amino acid	<i>C. acutus</i> muscle (1)	Beef liver (2)	CS	Chicken meat (2)	CS	Chicken carcass (3)	CS	Piglet carcass (3)	CS	<i>C. niloticus</i> muscle (4)	CS
Threonine	5.33	4.26	0.80*	3.78	0.71*	4.24	0.80*	3.94	0.74	4.23	0.79*
Isoleucine	5.24	4.74	0.90*	4.09	0.78	4.09	0.78*	3.30	0.63*	4.54	0.87*
Leucine	8.87	9.38	1.06	6.9	0.78	7.25	0.82	7.14	0.80	8.23	0.93
Lysine	9.84	7.89	0.80*	6.7	0.68*	7.2	0.73*	5.14	0.52*	8.84	0.90
Valine	4.82	6.19	1.28	4.7	0.93	4.4	0.91	4.82	1.00	4.44	0.92
Arginine	5.88	6.09	1.04	5.45	0.93	6.68	1.14	7.33	1.25	8.12	1.38
Histidine	2.99	3.08	1.03	2.95	0.99	2.62	0.88	2.14	0.72*	2.74	0.92
Methionine	2.93	2.66	0.91	2.03	0.69*	2.74	0.94	2.80	0.96	2.66	0.91
Phenylalanine	4.45	5.32	1.20	3.56	0.80	4.34	0.98	4.41	0.99	3.72	0.84*
Tryptophan	0.59	1.29	2.19	1.24	2.10	1.02	1.74	0.82	1.39	ND	

Table 3. Essential amino acid profile (EAA) of *Crocodylus acutus* muscle, and chemical score (CS) of fish protein muscle, used or proposed in its diet (g of amino acids per 100 g of protein). *Limiting amino acids. 1) Centro de Investigación en Alimentación y Desarrollo (CIAD) (México), 2) USDA (2017), 3) Pinheiro & Lavorenti (2001).

Amino acid	<i>C. acutus</i> (1)	Tilapia (whole) (2)	CS	Catfish (3)	CS	Tuna (3)	CS
Threonine	5.33	4.46	0.83	4.51	0.85*	4.38	0.82*
Isoleucine	5.24	3.97	0.76*	4.45	0.85*	4.6	0.88*
Leucine	8.87	6.97	0.79	7.57	0.85	8.12	0.92
Lysine	9.84	7.44	0.76*	9.10	0.92	9.18	0.93
Valine	4.82	4.76	0.99	4.84	1.01	5.15	1.07
Arginine	5.88	6.80	1.16	6.24	1.06	5.98	1.02
Histidine	2.99	1.91	0.64*	2.19	0.73*	2.94	0.98
Methionine	2.93	3.21	1.10	2.92	1.00	2.95	1.01
Phenylalanine	4.45	4.04	0.91	3.98	0.90	3.9	0.88*
Tryptophan	0.59	0.80	1.37	1.19	2.03	1.11	1.89

to the catfish for threonine, isoleucine, and leucine. In tuna, threonine, isoleucine, and phenylalanine also cover about 80% of the requirements for the crocodile.

Table 4 shows the chemical score calculated for vegetable sources. Methionine is the first LAA; second and third were lysine and threonine. Lysine is the first LAA in wheat and corn meal, although the AA profiles differ from crocodile in several AA. The second and third LAA for wheat meal were methionine and threonine. For corn meal, the second and third LAA were threonine and isoleucine. The scores of the first LAA from plants are significantly lower than in animals.

DISCUSSION

This is the first report on the muscle AA profile of *C. acutus*. The information allows, not only to obtain the EAA requirements of this species, but the possibility to

formulate diets, on a scientific basis, including the possibility of incorporating suitable vegetable protein sources. It also provides information on protein quality of crocodile meat. In general, it shows that EAA profiles of animal protein sources best fit the profile of EAA for *C. acutus*. Among the terrestrial animal protein sources, beef liver is the most similar. Among fish, there are very good sources of some EAA for the crocodile, so they can be supplemented with ingredients of terrestrial animals. Pinheiro & Lavorenti (2001) studied the growth of offspring of the broad-snouted caiman *Caiman latirostris* (Daudin, 1802) with different sources of animal protein (chicken and pig carcasses, whole tilapia, and a mixture of the three) and found no difference in weight gain, but differences in size (total length). However, the EAA profile of alligator muscle was not determined; therefore, it is not possible to determine if the diets fulfilled the caiman's AA requirements.

Table 4. Essential amino acid profile (EAA) of *Crocodylus acutus* muscle, and chemical score (CS) of protein of cereals commonly used in animal nutrition (g amino acids per 100 g protein). *Limiting amino acids. 1) Centro de Investigación en Alimentación y Desarrollo (CIAD) (México), 2) USDA (2017).

Amino acid	<i>C. acutus</i> (1)	Soy meal (2)	CS	Wheat meal (2)	CS	Corn meal (2)	CS
Threonine	5.33	3.88	0.73*	2.7	0.51*	3.75	0.70*
Isoleucine	5.24	4.51	0.86	3.35	0.64	3.58	0.68*
Leucine	8.87	7.58	0.85	6.79	0.77	12.6	1.38
Lysine	9.84	6.19	0.63*	2.71	0.28*	2.8	0.28*
Valine	4.82	4.72	0.98	4.26	0.88	5.06	1.05
Arginine	5.88	7.31	1.24	4.9	0.83	4.98	0.85
Histidine	2.99	2.85	0.95	2.7	0.90	3.05	1.02
Methionine	2.93	1.34	0.46*	1.72	0.59*	2.09	0.71
Phenylalanine	4.45	5.18	1.16	5.16	1.16	4.91	1.10
Tryptophan	0.59	1.34	2.27	1.31	2.22	0.70	1.19

Soybean possess an amino acid profile that implies a potential suitability as a partial substitute for animal-based meals, although it would be necessary to establish an optimal level. For *Caiman crocodilus* (Linnaeus, 1758) and *Alligator mississippiensis* (Daudin, 1802), Herbert & Coulson (1975) state that the corn protein zein (raw, cooked, or autoclaved) and wheat, pea, and soybean flour for meal are not digestible; therefore, does not increase the concentration of free AA in reptilian plasma. However, casein and fish muscle were digested quickly, but only a small increase in free AA concentrations was found in plasma. Koza *et al.* (2010) in studies with “yacaré” alligator (*Caiman latirostris*, Daudin, 1802), found that diet A (pellet and beef meat) containing higher proportion of fats, produced significant elevations in triglycerides, magnesium and potassium, and the diet B (pellets and chicken meat), containing higher proportion of protein and minerals, caused significant increases in body weight, cloaca-vent length, head length, mean corpuscular hemoglobin concentration, sodium, total cholesterol, HDL-C and LDL-C and conclude that changes allow the use of both diets for this species.

Beyeler (2011) studied requirements of juvenile *C. niloticus* and determined that diets with high percentages of animal protein, such as ground raw chicken, are more digestible than those containing high percentages of vegetable protein (soy) and found a directly proportional effect on growth. In the same study, the author analyzed for AA in the tested diets; however, the LAA were not calculated, although the EAA profile of the Nile crocodile had been reported by Hoffman *et al.* (2000). These authors mention that Nile crocodile farms produce skins and meat. Surplus meat is used to fatten younger crocodiles. The strategy of supplementing the diet of crocodiles in captivity with corpses of dead crocodiles of the same species seems to

be effective, since they engage in cannibalism in the wild. The above considering that the muscle of the same species would be theoretically the ideal to satisfy the requirements of EAA.

In the case of *C. acutus* in Mexico, there are no commercial farms, but there are breeding facilities to increase declining populations. Satisfactory diets are important to reach this objective. Hernandez-Hurtado *et al.* (2012) showed that diets consisting of a mixture of the horse jact *Caranx caballus* (Günther, 1868) and tuna *Euthynnus affinis* (Cantor, 1849) and beef liver allow proper growth of *C. acutus* offspring in captivity. This coincides with the results obtained in the present study. The amino acid deficiencies of a protein can be remedied by complementing proteins with different LAA, or by supplementing with the purified LAA.

CONCLUSIONS

The EAA profile of *C. acutus* muscle and the CS of protein sources may suggest an optimal diet, preferably supplemented with marine fish and terrestrial animal ingredients, such as beef liver. Our results suggest that both protein sources provide EAA similar to the AA profile of *C. acutus* muscle. The possibility of including carcasses of dead crocodiles is not illogical and should be considered as an additional supplement to conventional diet. In contrast, the integration of plant proteins, does not appear to be a practical alternative given the reported deficiencies in several EAA, particularly methionine, lysine, and threonine.

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