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Reptile Bushmeat, an Alternative for the Supply of High Biological Value Proteins?

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Abstract: The sustainable use of wildlife is considered a tool for conservation in addition to generating benefits for the local population. Commercial reptile hunting targets skins, generating other by-products such as meat and fat. Meat from hunted reptiles is widely available in localities where management plans are in place and is evaluated as easily accessible for hunters and their families. The objective of our research was to evaluate the percent composition, protein composition and microbiological quality of black and white tegu and yellow anaconda meat. For this study, we obtained meat samples of both species from wild specimens. The composition of both meats showed a proportion of moisture greater than 70%, a good proportion of protein (around 20%) and a low proportion of intramuscular fat (<2%). In the meat of black and white tegu, we found all of the essential amino acids in the recommended proportions, while the meat of yellow anaconda tryptophan did not reach the recommended levels to meet adult requirements. Both meats had good microbiological quality and were free from pathogenic bacteria. The results obtained reveal distinctive nutritional qualities of the meats analyzed, which can be recommended as an alternative and/or complementary source of good quality protein for human consumption.

Keywords: *Salvator merianae*; *Eunectes notaeus*; amino acids; chemical score

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1. Introduction

The sustainable use and economic exploitation of wildlife are possible through different management plans that favor the conservation of species and their habitats in addition to benefiting local inhabitants [1]. In Argentina, we can mention examples of sustainable use and conservation programs based on hunting techniques with more than 20 years of work, such as Proyecto Curiyú and Proyecto Tupinambis, which make the sustainable use of yellow anaconda (*Eunectes notaeus)* and black and white tegu possible (*Salvator merianae*, ex *Tupinambis merianae*), respectively [2,3]. These reptile species are included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and, therefore, their hunting and trade are regulated by the previously mentioned conservation programs. Both species are listed as "least concern" according to IUCN's Conservation criteria of biodiversity [4,5].

Yellow anaconda and black and white tegu are hunted for their skins, which are destined for export, but the meat of these species is scarcely exploited. Generally, wild species' meat and fat are little consumed, partly due to a lack of knowledge of their nutritional and organoleptic characteristics. In this context, numerous studies show the positive nutritional characteristics of the meat and fat of some other reptile species. For example, both *Caiman latirostris* and *Iguana iguana* meats have a high protein percentage and can be considered as an alternative source of animal protein with good nutritional quality for human consumption [6–11]. There are also reports on reptiles' fat, such as *C. latirostris* and *S. merinae*, which mention their potential value for food since they contain a high percentage of n-3 polyunsaturated fatty acids and recommended nutritional indexes [12,13].

There is currently a clear interest in both the domestic and the international market for alternative or exotic meats, resulting in a commercial opportunity for both the programs and the local people who hunt these species [14–16]. This encourages the evaluation of reptile meats from sustainable use management plans. It should be noted that these meats are currently not used or have no specific use.

The role of meat as a source of protein is unequivocal, but its nutritional content can vary substantially [17]. Therefore, the evaluation of protein quality is recommended to establish its nutritional quality. Another important point to evaluate in meats is their microbiological quality since many diseases are foodborne, caused by pathogens [18]. This is because meat's characteristics (high percentage of moisture, protein, peptide and amino acid supply) constitute an ideal medium for the growth of microorganisms [19,20]. Therefore, sanitary entities determine microbiological quality standards that meat products must meet to be considered fit for consumption without endangering the health of individuals.

In this study, we evaluated the percent composition, protein composition (in terms of amino acids quantity) and microbiological quality of the meats of two wild species, yellow anaconda and black and white tegu, in order to determine their nutritional quality and suitability for human consumption. In addition, we predicted the protein quality of meats through their chemical score using a pattern of amino acids according to human requirements.

2. Materials and Methods

We used muscle tissue samples from wild individuals from hunting programs "Proyecto Curiyú" in Formosa province and "Proyecto Tupinambis" in Santa Fe province (Argentina), both programs supported by provincial and national authorities (Resolutions DISP241 PR5579; 561/93; 216/96 and 1437/00). The animals were killed according to the protocols of the programs [2,3]. Briefly, the killing consists of an immediate loss of consciousness as a result of a slight blow to the head and subsequent separation of the brain and the spinal cord through the introduction of a sharp element from the back of the skull to the front, guaranteeing the destruction of the brain. This method is recommended for the killing of reptiles [21]. After death, the skin is removed mechanically, detaching it from the muscle. Once the skin has been obtained, it is eviscerated in order to obtain the carcass. Yellow anaconda carcasses were harvested by hunters in a single operation.

For black and white tegu, we collected meat samples in different operations under different work protocols. In the first instance, we took samples under the traditional methodology and conditions of the local people, and in the second instance, we obtained samples after implementing recommendations to avoid cross-contamination that could occur when removing the skin and eviscerating the animals. Although the hunters were already complying with some of our suggestions, we decided to detail a systematized series of steps with an emphasis on the critical points where there is considered to be a greater likelihood of contamination of the carcass. Details are as follows: 1. Wash hands and clean the knife before starting to work on each individual); 2. Before removing the skin, clean the animal's hide with a damp cloth and then disinfect it with sodium hypochlorite diluted in water, using the concentration suggested by the WHO for surfaces in contact with food: 0.05–0.1% (500 to 1000 ppm); 3. Be careful not to pierce viscera (to prevent the contents of the viscera from contaminating the meat); 4. If the individual spills urine or feces during the dressing and evisceration process, the work area must be sanitized and disinfected in order to continue. The objective was to record and compare the microbiological analysis parameters obtained in both scenarios in order to propose the best management practices for obtaining meat fit for human consumption.

We obtained the carcasses of five individuals of yellow anaconda and ten individuals of black and white tegu (five in each meat sampling protocol), which we kept at −18 °C until they were processed in the laboratory. We homogenized the meat samples from each animal in the laboratory and separated portions of approximately 20 g to analyze the meat microbiologically and determine its percent composition (percentage of moisture, protein, ash and fat). In addition, we evaluated the quality of the protein provided by the meats. One of the main factors determining protein quality is the proportion of essential amino acids (EAAs) and non-essential amino acids (NEAAs) provided by the protein [22]. Amino acids (AAs) that cannot be synthesized by humans and other mammals and, therefore, must be supplied by the diet, are considered essential [23]. Consequently, we determined the AAs present in the meats of yellow anaconda and black and white tegu and the proportion of each of them.

We proceeded as follows:

- To analyze the microbiological quality of the meats we determined the presence and quantified: Aerobic Mesophilic Bacteria, Total Coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*. and sulfite-reducing clostridia. The techniques proposed by Leiva et al. [13] were followed to determine them.
- Moisture percentage: we determined moisture content using the air-drying method at 125 °C for 4 h [24].
- Protein percentage: we performed automatic distillation using Kjeltec 2200 (Foss Tecator, Sweden). The samples were digested with sulfuric acid to convert organic nitrogen to ammonium ions. We obtained the ammonium ions by distillation and then titrated them with hydrochloric acid until neutralization [24]. The nitrogen content was multiplied by a factor of 6.25 to obtain the protein content of the meat. The results are expressed in g of fat/100 g of meat.
- Fat percentage: we used a Soxtec 2055 (Foss Tecator, Sweden). We extracted soluble material from the dry samples by a two-step treatment with petroleum ether solvent. We removed the solvent by condensation and determined the dry weight of the extracted soluble material after drying [24]. The results were expressed as g protein/100 g meat.
- Ash percentage: we determined this after drying the meat at 525 °C, according to [24].
- Amino acids percentage: we hydrolyzed the protein and identified the hydrolysates according to the procedure described by Alaiz [24]. The results were expressed in g of AA/100 g of protein.

The protein quality of the meats was assessed from the calculation of the Chemical Score (CSs). This method detects the EAA that are present, where the lowest value is that defined as “limiting amino acid,” since it limits the quality of the protein under study and establishes the efficiency with which dietary nitrogen can meet the EAA requirements [17,25]. For its calculation, we used the most recent standard protein for adults proposed by FAO [26]. In addition, the Index of Essential Amino Acids (IEAAs) [27] was determined.

$$\mathrm{CS} = \frac{\text{mg of EAA in g of study protein}}{\text{mg of EAA in g of reference protein}} \times 100 \tag{1}$$

$$\mathrm{IAAE} = 100 \times \sqrt[n]{\frac{a}{a_p} \times \frac{b}{b_p} \times \frac{c}{c_p} \ldots\ldots \times \frac{j}{j_p}} \tag{2}$$

a, b, c, j: essential amino acid content of the protein under study.

a_p, b_p, c_p, j_p: content of essential amino acids in the standard protein of FAO/WHO/UNU [26].

n: number of amino acids under study.

3. Results

In the microbiological analysis of yellow anaconda meat, we found that the counts of *Total Aerobic Mesophilic Bacteria, Total Coliforms, Escherichia coli, Staphylococcus aureus, Salmonella spp*. and sulphite-reducing clostridia are below the maximum values allowed by the National Administration of Medicines, Food and Medical Technology (ANMAT) [28]. In meat samples of black and white tegu obtained from butchering with traditional hunter protocols, *Staphylococcus aureus* counts were found to exceed the values permitted by the ANMAT [28]. While the samples obtained from the implementation of the proposed butchering recommendations were found to be microbiologically fit for consumption as the counts of all microorganisms studied were below the maximum acceptable limits (Table 1).

Table 1. Maximum average values determined in 1 g in the meat of yellow anaconda and black and white tegu and their comparison with the standards required for fresh meat, frozen meat and ground meat (ANMAT) for each of the microorganisms evaluated in the microbiological quality analysis for human consumption.

Microorganisms	Determination of Yellow Anaconda Meat	Determination of Black and White Tegu Meat Obtained from Traditional Butchering	Determination of Black and White Tegu Meat Obtained from Butchering with Recommendations	Recommended Values
Total *mesophilic aerobic bacteria* (CFU/mL)	<52/g	5600/g	2934/g	<100,000
Total *coliform bacteria* (CFU/mL)	<10/g	4/g	4/g	<250
Determination of *Escherichia coli* (CFU/mL)	<0.4/g	0.32/g	9.36/g	50
Determination of *Staphylococcus aureus* (CFU/mL)	<100 (4/g)	30,000 (1200/g) *	1625 (65/g)	50 to 100
Determination of *Salmonella* sp.	Absent	Absent	Absent	Absent

* The values that exceed the maximum limit for human consumption recommended by ANMAT.

In the percent composition of yellow anaconda and black and white tegu meats, we observed that both meats have approximately at least 20% protein, exceed 70% moisture and are low in fat content (<2%) (Table 2). In addition, we identified eight EAAs and ten NEAAs in the samples of both meats (Table 3). Of the EAAs, the highest values were observed for methionine in yellow anaconda meat and lysine in black and white tegu meat. In the meat of both species, the tryptophan EAA had the lowest value. As for NEAAs, we identified the highest values for glutamic in yellow anaconda and aspartic in black and white tegu. The lowest NEAA value was found for cysteine in both meats.

Table 2. Percent composition of the meats of the species studied expressed in % (mean ± standard deviation).

	Moisture (%)	Proteins (%)	Fats (%)	Ashes (%)
Yellow anaconda	79.15 ± 0.39	19.21 ± 1.12	0.33 ± 0.17	0.92 ± 0.03
Black and white tegu	71.35 ± 1.15	26.02 ± 1.07	1.45 ± 0.82	1.37 ± 0.25

Table 3. Amino acid content of meat in the species studied expressed in g amino acid/100 g protein (mean ± standard deviation).

Amino Acids	Yellow Anaconda	Black and White Tegu	Beef [a]	Pork [a]	Chicken [b]
Essentials					
Lysine	9.4 ± 0.6	9.6 ± 0.5	8.2	7.9	7.2
Leucine	8.4 ± 0.4	8.9 ± 0.6	8.5	7.6	7.2
Isoleucine	4.3 ± 0.1	4.7 ± 0.3	5.0	4.8	4.5
Threonine	4.6 ± 0.3	5.3 ± 0.3	4.2	5.2	4.6
Methionine	11.2 ± 3.0	8.9 ± 3.7	2.2	2.6	1.5
Tryptophan	0.3 ± 0.1	0.7 ± 0.4	1.3	1.5	1.5
Phenylalanine	4.3 ± 0.2	4.4 ± 0.2	4.1	4.3	3.6
Valine	4.0 ± 0.1	4.6 ± 0.4	5.6	5.2	4.7
Non essentials					
Arginine	7.0 ± 0.4	7.4 ± 0.3	6.4	6.6	6.8
Proline	1.8 ± 0.3	2.5 ± 0.8	5.2	4.4	4.1
Glutamic acid	17.8 ± 5.1	10.9 ± 1.9	14.3	14.6	13.7
Aspartic acid	16.2 ± 1.9	17.7 ± 0.6	8.9	8.8	8.7
Cysteine	0.9 ± 0.1	1.1 ± 0.1	1.5	1.2	0.92
Glycine	4.7 ± 0.5	6.0 ± 0.3	7.2	6.0	6.5
Tyrosine	3.6 ± 0.4	3.6 ± 0.4	3.3	3.1	2.7
Serine	8.2 ± 0.5	10.1 ± 1.5	3.9	4.1	3.3
Alanine	5.9 ± 0.4	6.5 ± 0.5	6.3	6.4	5.8
Histidine	3.8 ± 0.4	4.4 ± 0.9	2.8	3.1	2

[a] Amino acid values extracted from Ahmad [29]. [b] Amino acid values extracted from Verback [30].

Once the amount of AA in the meat of both species studied had been determined, we evaluated the protein quality, taking into account the reference protein proposed by FAO [26] (Table 4); this is a theoretically existing protein whose composition is adequate to correctly satisfy the needs of human beings [31]. Based on the standard protein, the CS of the EAA present in the meats studied was calculated as a correlation parameter of the biological value of the proteins according to the formula referred to in the methodology. In black and white tegu, the CS of all EEAs was greater than 100 and in yellow anaconda two of the EEAs were below 100 (tryptophan, CS: 49.2 and valine, CS: 99.63) (Table 4).

Table 4. Chemical score of essential amino acids (mean ± standard deviation) for both meats under study and for traditionally consumed meats (values from Table 3 were used for calculation), in relation to the standard protein of amino acid score recommended for older children, teenagers and adults [26].

Amino Acids	Scoring Pattern AA [a]	Chemical Score Yellow Anaconda	Chemical Score Black and White Tegu	Chemical Score BEEF	Chemical Score Pork	Chemical Score Chicken
Isoleucine	3.0	143.0 ± 1.4	157.0 ± 8.5	166.7	160	150
Leucine	6.1	137.1 ± 7.2	146.4 ± 9	139.3	124.6	118
Lysine	4.8	196.6 ± 11.6	200.2 ± 10.6	170.8	164.6	150
Methionine + Cystine [b]	2.3	527.8 ± 134.7	432.2 ± 163.6	160.9	165.2	105
Phenylalanine + Tyrosine [b]	4.1	191.2 ± 14.1	193.2 ± 14.5	180.5	180.5	153.7
Threonine	2.5	183.8 ± 11.6	211.2 ± 13.6	168	208	184
Tryptophan	0.66	49.2 ± 5.4	103.8 ± 58.9	197	227.3	227.3
Valine	4.0	99.6 ± 3	115.4 ± 9.4	140	130	117.5
IEAA		155.8 ± 12.8	174.5 ± 13.1	164.4	166.9	136.5

AA scoring standard: amino acid values recommended by FAO to cover nutritional requirements in older children, teenagers and adults. Expressed in g of amino acid/100 g of protein. IEAA (Index of Essential Amino Acids, obtained according to Shahidi and Synowiecki). [a] Amino acid values of scoring pattern extracted from FAO [26]. [b] These amino acids are considered in pairs as methionine is a precursor of cysteine and phenylalanine of tyrosine.

4. Discussions

Bushmeat can contribute to an alternative source of essential nutrients, especially in rural households where access to safe and nutritious food is limited. However, scientific information on yields, quality and nutritional content is needed to promote the consumption and marketing of this type of meat [14]. One of the main concerns of the scientific community is the potential diseases transmitted by handling and consuming wild foods [32]. Regarding this, our study shows that the yellow anaconda meat obtained during butchering presented microbiological parameters in accordance with the values permitted by ANMAT, making it fit for human consumption. This shows that the butchering practices carried out by the local people are adequate to obtain a meat product suitable for safe use. However, black and white tegu meat obtained by hunter's traditional butchering practices, *Staphylococcus aureus* counts exceeded the maximum permissible limits for consumption. It should be noted that *S. aureus* is one of the most frequently found pathogens in food [33,34]. This microorganism can be carried in the nose or hands of food handlers, as well as on the skin, gut and nose of animals [35,36]. Subcutaneous muscle tissue (essentially sterile in alive and healthy animals) can become contaminated very quickly as a result of the spread of normal microbiota from the animal's skin and intestines during butchering and from the butcher's house environment [37].

Since the main product obtained from reptiles is their skin, hunters remove the skin with care, trying to guarantee and preserve its quality, thereby increasing the amount of handling and the risk of contaminating the carcass [38,39]. Therefore, by reducing the bacterial load on the skin, contamination of the muscle during butchering will be reduced [40]. Hence, the main recommendation to hunters was to sanitize and disinfect the skin prior to butchering. Disinfection was carried out with a 0.05 to 0.1% (500 to 1000 ppm) dilution of bleach water recommended by the WHO for food contact surfaces [41]. The bleach water is a sodium hypochlorite solution, and this disinfectant was chosen as it is easily accessible and inexpensive for hunters. Samples of black and white tegu taken after applying the recommendations showed a reduction in the counts of microorganisms present in the meat. We obtained microbiological values below the maximum limit allowed by health authorities, including *S. areus*. It is evident that reinforcing hygienic handling practices is necessary to obtain meat fit for human consumption, thus ensuring optimal levels of food safety. It should be noted that the reptile species under study have very different body structures; black and white tegu requires a lot of handling during skin removal due to the presence of folds and limbs, whereas yellow anaconda does not have such obstacles, thus reducing the risk of contamination from the skin to meat.

In relation to the nutritional value, our results showed that the meats of both reptile species studied contain a percentage of protein similar to the content of traditionally consumed meats, such as beef (22.8 g/100 g of meat) and chicken meat (21.4 g/100 g of meat) [42]. In particular, black and white tegu meat exceeded these protein values (26 g/100 g meat).

If we consider the nutritional value of the proteins in terms of their EAA composition, both yellow anaconda and black and white tegu bushmeats were found to contain all eight EAAs and their proportions were similar to those of beef, pork and chicken, with the exception of tryptophan content, where a much lower contribution was observed in bushmeats (Table 3). Black and white tegu proteins, like beef, pork and chicken, contained all EAAs in adequate proportions according to the FAO [26] standard: CS $\geq$ 100 (Table 4). Therefore, the meat of this species meets the recommended nutritional requirements for adults.

We found that yellow anaconda meat proteins were characterized by a limited protein nutritional value in the amount of tryptophan (CS = 49.24, half of the minimum required value), and although valine did not reach the minimum value, it was very close to

100 (CS = 99.63). From a food and dietary point of view, the protein content of yellow anaconda meat is classified as "incomplete" as it does not meet the recommended daily requirement of tryptophan. Tryptophan deficiencies are associated with pellagra and psychological, cognitive and behavioral disorders, such as dementia and depression [43]. Several dietary proteins have been described as good sources of tryptophan, such as eggs, cow's milk and chicken meat, foods that would be available to local people [22]. It would therefore be advisable for the consumption of yellow anaconda meat to be complemented by the previously mentioned foods, as this would provide all the EAA necessary for the human body, thus achieving a balanced pattern of AA [31].

The fat content of yellow anaconda and black and white tegu meats was less than 2%, clearly indicating that they are lean meats compared to traditionally consumed meats such as chicken (14.07 g/100 g) and beef (5.93 g/100 g) [42]. Although being considered lean, reptile meats have an interesting fatty acid profile since they provide a good proportion of n-3 polyunsaturated fatty acids [8,44]. A "healthy meat" is considered to be one that contains high levels of protein and low levels of fat, being these last ones recommended to have a higher percentage of n-3 polyunsaturated fatty acids in relation to saturated fatty acids [45]. This classification highlights the nutritional properties of these reptile meats in terms of their nutritional composition, as they meet most of the above-mentioned characteristics, making them very good alternatives for hunters and their families, as well as looking good and being easy to prepare and consume [46]. Another critical point is the sensory characteristics, which acceptability to the consumer has been tested, obtaining positive results, but not yet been published. Both meats showed acceptability rather than indifference, but the anaconda meat was tastier to consumers (as shown in a previously mentioned work). In addition, yellow anaconda meat showed a similar acceptability value to pork and was described as rich, juicy and tasty in consumer tests [46].

Our study demonstrated that yellow anaconda and black and white tegu can be a high nutritional quality protein alternative. This allows us to think about its future commercialization with the aim of increasing the economic return both for the people involved in the sustainable use programs and for the programs themselves. However, it is important to recognize that the reptile meats evaluated in this work are far from being produced in large volumes and from being able to replace other commercial meats. We can mention that in the last 10 years in the province of Formosa, within the framework of the "Proyecto Curiyú", an average of approximately 3800 yellow anaconda skins have been collected annually. The estimated annual meat production, which exceeds 13 tons, could be used by the local population, either directly for consumption or indirectly through commercialization. In the case of black and white tegu in the provinces with hunting permits, 125,000 hides were collected during the 2021–2022 harvest, which could have produced 120 tons of meat. It is important to stress that the hunting of yellow anaconda and black and white tegu, like other native species, is not indiscriminate but is regulated under national and provincial regulations that limit their export and commercialization in order to favor the conservation of the species and their habitat [2,3].

In conclusion, the use of bushmeat from the sustainable use programs of two reptile species, yellow anaconda and black and white tegu, can improve nutrition for hunters and their families. This is because this is an alternative food source of animal origin, with high protein, low-fat content, good amino acid profile and microbiologically safe.

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