

Evidence-based practice

A novel protocol for hand-rearing of the toco toucan *Ramphastos toco* using a non-commercial diet based on native ingredients

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Abstract

The toco toucan *Ramphastos toco* was considered extinct in the Tucuman Yungas (central region of Argentina) until 2011, when it was recorded again; however, the species remains threatened. The Parque de la Biodiversidad (Formerly Cordoba Zoo) is an institution committed to conservation and has a specific project for toucan conservation, which includes the breeding and eventual release of captive-reared toucans in the forests of Tucuman. This article describes a novel protocol for artificial incubation and rearing of the chicks until their release age. Eggs were incubated at 37°C and 45% humidity, rotating them four times a day. Forty-eight hours before the expected hatching, humidity was increased to 60% and rotation was stopped. Embryo development occurred as naturally expected, and five out of six eggs successfully hatched. During the first three days of life, the chicks were kept under the same conditions as those during the incubation period. Thereafter, the temperature decreased by 1°C per day, reaching 31°C by the eighth day of life. The chicks exhibited a proper and vigorous response to external stimuli, comparable to that of their wild-reared counterparts. Five toucan chicks were successfully hand-reared to 60 days of age using the proposed formula and rearing protocol, before being transferred to an alternative facility for eventual soft-release into the wild.

Background

Toucans are birds endemic to the Neotropical region that are characterized by their large prominent bills. They primarily inhabit forested areas and are predominantly frugivorous. The toco toucan *Ramphastos toco*, also known as the giant toucan, is the largest species within the family Ramphastidae. Its geographical range extends from the Guianas to northern Argentina, the latter country representing the southernmost limit of its distribution. In Argentina, this species occurs in the forests and woodlands of the north (Babarskas et al 1995; Narosky and Yzurieta 2003), exhibiting two distinct ecotypes that are currently recognized as subspecies: *R. t. toco* in the northwest (Salta, Jujuy, and Tucumán provinces) and *R. t. albogularis* (Cabani 1862) in the northeast (Chaco, Formosa,

Santa Fe, Corrientes, and Misiones provinces) (de la Peña 1999).

Regionally, the distribution of the toco toucan is strongly tied to the Piedmontane Forest in the Tucumán province, which represents a critical habitat utilized by the species for altitudinal seasonal movements (Vides Almonacid et al. 1998). Much of this habitat was lost during the late 1990s, and given the scarcity of additional reports and the antiquity of past records, recent evaluations strongly suggested that the species was considered extirpated from the province (Brandán and Navarro 2009).

However, on 14 September 2010, two individuals were sighted in flight, crossing the San Javier River channel from the eastern to the western bank (Ortiz et al. 2011). In this area, the San Javier River flows from north to south along the La

Sala Valley, west of the homonymous mountain range. From a phytogeographical perspective, the area belongs to the Yungas ecoregion, specifically within the Montane Forest belt (Brown and Grau 1993). This new record could be attributed either to an increase in regional relict populations or to a recolonization process facilitated by habitat recovery in sectors of the Tucumán Yungas, particularly within the Sierra de San Javier (Grau et al. 2010; Nuñez Montellano et al. 2010; Ortiz et al. 2011).

Toucans are primarily frugivorous, with a preference for large, lipid-rich fruits (Galetti et al. 2000; Chaves-Campos 2004; Ragusa-Netto 2008; Dos Santos and Ragusa-Netto 2013; Silva 2019). Despite their frugivorous dependence, they also consume large arthropods and small vertebrates, as well as opportunistically raiding other birds' nests (Skutch 1971; Remsen et al. 1993; Sick 1997; Short and Horne 2002; Moroni et al. 2017). Avian resource predation (eggs, nestlings, and small adult birds) has been shown to increase between October and December, coinciding with the toucan's breeding season (Oliveira and Tubelis 2023). Both parents share chick-rearing responsibilities, alternating between foraging efforts and nest guarding (Vincent 2007).

The conservation status of the toco toucan is severely affected by habitat loss driven by deforestation and land-use changes (Chébez 2009). Additionally, individuals are frequently captured and illegally traded as exotic pets, leading to their inclusion in CITES Appendix II. Although global populations are estimated to have declined significantly, the toco toucan is currently classified as "Least Concern" by the International Union for Conservation of Nature (IUCN) (BirdLife International 2017), despite a projected population decline of over 30% over ten years or three generations at regional scales (Ruggera et al. 2022).

For an increasing number of taxa, ex-situ conservation management plays a crucial role in preventing extinction (IUCN/

SSC 2014). Captive propagation of threatened species serves as an ex-situ strategy to establish genetic reservoirs and provide young individuals for wild population reinforcement programs. To address these modern zoological challenges, an assisted reproduction project for the toco toucan was established at the Parque de la Biodiversidad (Córdoba, Argentina), utilizing a resident adult pair.

Significant gaps persist regarding the ex-situ breeding of the toco toucan. Nonetheless, practical hand-rearing protocols have been developed (Short and Horne 2002; Vince 2020; Terme et al. 2023). At Parque de la Biodiversidad, assisted rearing became mandatory due to the parental pair's failure to exhibit appropriate husbandry behaviors. Consequently, this study aims to document a comprehensive protocol for captive hand-rearing of toco toucans, evaluating a handmade, non-commercial diet formulated from native and natural ingredients.

Action

Incubation and Animal Husbandry: Over two breeding seasons, the resident pair laid a total of six eggs. During the first season (January 2023), eggs were designated as p1 and p2. In the second season (December 2023), four eggs were laid and identified as p3, p4, p5, and p6. Egg p4 failed to hatch due to late-stage embryonic mortality, which was diagnosed via candling. This non-invasive technique involved assessing internal vascularization and embryo viability by placing a cold light source against the blunt pole of the egg (Hall et al. 2022).

Artificial incubation was conducted in a dedicated climate-controlled room, utilizing incubators calibrated to 37°C and 45% relative humidity (RH). RH was increased to 60% approximately 48 hours prior to expected hatching (Seibels 2001; Gualpa García 2022). This deviated from the parameters outlined by Miller and

Table 1. Homemade diet composition for hand-rearing of toco toucan chicks at Parque de la Biodiversidad

Period	Week 1	Week 2 and onwards
Ingredients	g per 100 g AF	g per 100 g AF
Papaya	68	64
Mouse pup (<1 week old)	31.2	
Baby chick > 1 week old		34.5
Monocalcium phosphate	0.36	1
Vitaraptor® Muda y cría	0.44	0.5
Nutritional profile	DM	DM
ME	2.959 kcal/g	1.388 kcal/g
CP	26.23%	34%
EE	7.59%	11.50%
CF	0	0.50%
Ca	1.81%	2.51%
P	1.12%	1.86%
Ca:P	1.6:1	1.3:1
Fe	152.389 mg/kg	62 mg/kg

ME:Metabolizable energy, CP: crude protein, EE: ether extract, CF crude fiber, Ca: Calcium , P: Phosphorus, Fe: Iron

Table 2. Developmental data from wild-reared and human-reared toucans

	Wild reared (Mikich et al. 2001)	Human reared (This report)
Incubation	15-16 days	17 days
Onset of self-feeding	56 days	50 days
Weaning	63-72 days	55-60 days
Feathering	48 days	50 days

Fowler (2015), who recommend incubating toucan eggs at 37.5°C and 55% RH; this adjustment was implemented to optimize egg weight loss under local ambient laboratory conditions.

Following hatching, chicks were transferred to a brooder set at 37°C and 45% RH. On day 3, the temperature was lowered to 34°C, and further reduced to 31°C on day 8. After an additional five days, the temperature was adjusted to 30°C and maintained until the chicks were fully fledged, followed by a progressive reduction to 27°C. While Miller and Fowler (2015) suggest transferring chicks to a hatcher at 37°C while gradually reducing temperature and increasing humidity up to 95%, this protocol maintained a strict 70% RH target.

Once fully fledged (approximately seven weeks of age), chicks were transitioned to ambient temperature. At approximately 48 days of age, they were moved to an indoor enclosure (0.5 m²) fitted with two separate perches spaced 60 cm apart and set

at a height of 20 cm from the floor to encourage initial muscle-strengthening exercises (as described in Miller and Fowler 2015). The final development stage took place in an outdoor flight aviary (30 m²) equipped with multi-level perches and obstacles for advanced flight training. This aviary was utilized during diurnal hours, while birds were housed in an indoor enclosure overnight.

Feeding Protocol

- Days 1–15 of age: During the first days of life, chicks were fed according to the blended formula detailed in Table 1 (first column). On day 1, the amount offered at each feeding corresponded to 5% of the chick's body mass, with a daily increase of 0.5% until reaching 10%. From the second week onwards, the second formula was implemented (Table 1, second column). The diet was administered every two hours between 08:00 and 20:00, resulting in seven daily feedings during the first 15 days. Following this period, feedings were reduced to five times a day between 08:00 and 18:00.

- Days 16–50 of age: Hand-feeding with small pieces of chopped fruit was introduced to facilitate the transition from the blended formula to solid food. A mixed ration consisting of 50% formula and 50% solid food was provided. By day 48, the chicks were fully transitioned to solid chopped food—maintaining the proportions of the blended formula—with three daily feedings and an increased intake of 13.3% per meal. During this transition process, various chopped fruits were gradually introduced on a weekly basis to diversify the diet. By approximately day 50, the chicks were feeding independently, maintaining the schedule of three daily meals.

- From 50 days of age onwards: Once the transition to a solid diet was complete, occasional environmental enrichment items—such as newborn mouse pups (“pinkies”), Zophobas larvae, and Tenebrio

Table 3. Average values and ranges of the blood profile of three toucan chicks *Ramphastos toco* prior to their relocation

Parameter	P3	P5	P6	Mean*	Range*	Unit
Urea	7.6	7.2	7.2	7.3	7.2 – 7.6	mg/dl
Creatinine	0.7	0.8	0.7	0.7	0.7 – 0.8	mg/dl
GPT/ALT	10.5	10.7	10.1	10.4	10.1 – 10.7	UI/L
GOT/AST	26.1	25.4	28.2	26.6	25.4 – 28.2	UI/L
FAS/ALP	47	49	51	49.0	47 – 51	UI/L
Tot. Protein	4.6	4.8	4.9	4.8	4.6 – 4.9	g/dl
Albumin	1.3	1.3	1.2	1.3	1.2 – 1.3	g/dl
Globulins	3.3	3.6	3.1	3.3	3.1 – 3.6	g/dl
Phosphorus	5.6	5.7	5.4	5.6	5.4 – 5.7	mg/dl
Calcium	9.2	9.2	9.3	9.2	9.2 – 9.3	mg/dl

*According to Cubas (2003). GPT: Glutamate-Pyruvate Transaminase, ALT: Alanine Aminotransferase, GOT: Glutamic Oxaloacetic Transaminase, AST: Aspartate Aminotransferase, FAS/ALP: Alkaline Phosphatase

Average mass and Growth rate

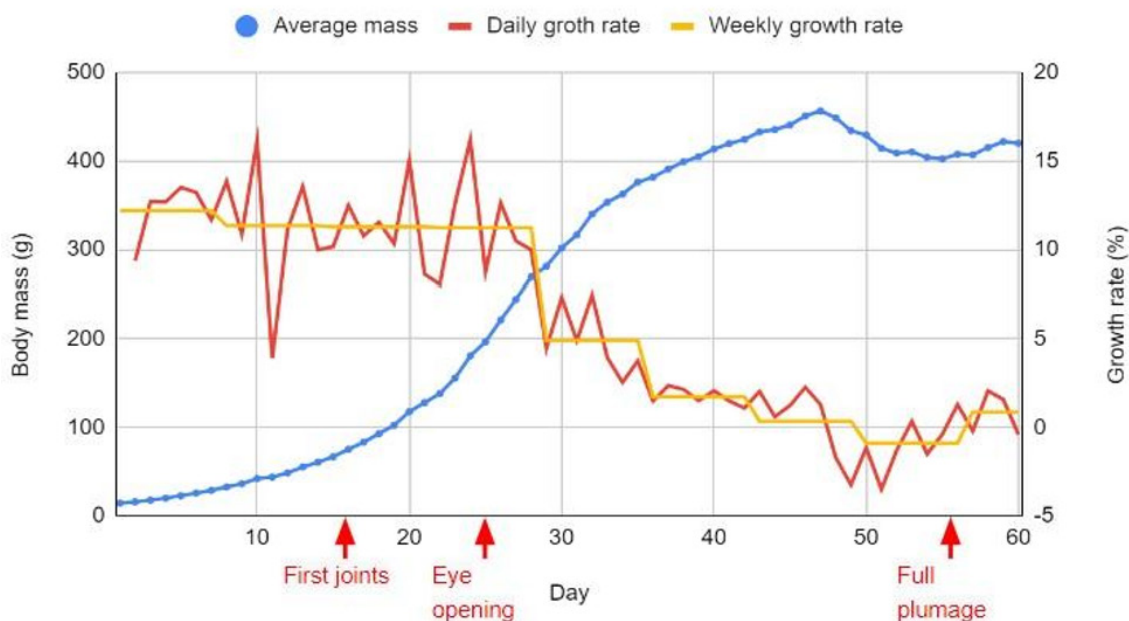


Figure 1. Average growth curve of the toco toucan *Ramphastos toco*

larvae—were introduced. These novel items were provided to ensure the birds retained dietary plasticity in accepting diverse food resources and to increase the quality of the offered protein.

Chicks were fed using a syringe for the blended formula until the crop was filled, and forceps were used for solid food. In both cases, feeding was delivered using a puppet that simulated an adult toucan, accompanied by background audio of conspecifics feeding nestlings. To prevent human imprinting, the personnel responsible for feeding wore surgical gowns to conceal the human silhouette, along with face masks and latex gloves.

Regarding initial nutrition, Miller and Fowler (2015) recommend a high-protein diet devoid of fruits or calcium supplements. Gage and Duerr (2007) suggest utilizing the commercial formula Exact Hand Feeding Formula-Macaw (Chilton®, Kaytee Products Inc.). In contrast, this study utilized a custom, homemade blended formula tailored to the specific nutritional requirements of the species (Dierenfeld and Graffam 1996; Cubas 2007; Leger et al. 2012), designed using Zootrition® software.

The diet formulation followed the recommendations of Leger et al. (2012), establishing energy and nutrient targets based on growth and development requirements. Protein content was maintained between 15% and 27%, and fat content ranged from 1% to 10% on a dry matter (DM) basis, consistent with recommendations for frugivorous birds with opportunistic animal protein intake (Dierenfeld and Graffam 1996). Fat levels were capped at 10% to mitigate the risks of hepatic damage and arteriosclerosis, as suggested by Cubas (2007).

Preventive Medicine

To prevent fungal infections (mycoses), prophylactic treatment of nystatin was administered twice daily during the first and final feedings. This protocol followed an alternating schedule of one

week of treatment followed by one week without treatment. A dose of 0.1 ml PO of nystatin was provided at each administration.

Blood Analysis

To evaluate the health status of the chicks, blood samples were collected from individuals designated as p3, p5, and p6 (2023–2024 cohort) at 138, 137, and 107 days of age, respectively. Venipuncture was performed on the brachial vein; however, the vessel collapsed easily, complicating the collection of adequate volume and sample quality for processing. The maximum volume collected was 0.5 ml per individual. One of the samples arrived at the laboratory slightly hemolyzed, which restricted the number of analytical parameters that could be evaluated. All sampling procedures were reviewed and supervised by the Veterinary Department and the Animal Welfare Department of Parque de la Biodiversidad.

Body Mass

To monitor growth dynamics over time, individual body mass was recorded daily during the first 60 days of life. From these data, the daily growth rate and the corresponding weekly averages were calculated.

Consequences

Incubation and Hand-Rearing

Over the course of the study, six eggs were incubated, five of which developed successfully and hatched, resulting in a hatching rate of 83%. In all cases, hatching occurred after 17 days of incubation. The five chicks originated from two clutches laid in consecutive years, and all individuals were successfully hand-reared using the previously described diet and rearing protocol.

Chick Development

Figure 1 illustrates the progression of the mean body mass of the five toucans. This growth curve has a sigmoidal shape, with the upper asymptote approaching 500 g, which corresponds to the average body mass of a conspecific adult. Daily growth values and their corresponding weekly averages are also integrated into Figure 1. The mean growth rate was 12.2% during the first week, 11.3% from the second to the fourth week inclusive, 4.9% during the fifth week, and 1.7% during the sixth week of life. From week six onwards, the growth rate became negligible, with values dropping below 1%.

The results of this study demonstrated a growth rate within the expected range for this species (5–15%), mirroring the growth curves described by Seibels (2001) and Gage and Duerr (2007) for hand-reared chicks. No substantial differences were observed when comparing the growth data from this study with published literature on toucan growth curves (Vince et al. 2007; Leger et al. 2012; Miller and Fowler 2015; Terme et al. 2023).

Incubation lasted 17 days at Parque de la Biodiversidad, compared to the 18 days reported by Vince et al. (2007) and Miller and Fowler (2015). Other developmental milestones, such as pin feather emergence, eye-opening, and the onset of solid food consumption, were highly comparable. However, nutritional weaning was achieved at 55–60 days, in marked contrast to the 70–84 days reported by Vince et al. (2007) and the 65 days described by Terme et al. (2023) for hand-reared toucans. On average, feather sheath emergence occurred at day 14, eye-opening at approximately day 20, complete plumage development at a mean of 48 days, and self-feeding at day 50.

Developmental data from wild toucans, or those reared by parents under human care, were also comparable: 15–16 days of incubation versus 17 days in this study; the onset of self-feeding at day 56 compared to day 50 in this protocol; and weaning between 63 and 72 days, as opposed to the 55–60 days observed here. Complete fledging was recorded between days 43 and 50, which is fully consistent with previously reported values (see Table 2).

Blood Analysis

The blood data obtained are summarized in Table 3. As previously mentioned, only a limited set of parameters was evaluated due to the technical difficulties encountered during venipuncture. The analysis revealed values well within the established reference range for normal chicks (Cubas 2007).

Previous attempts to rear toucans in captivity within Argentine zoos and bioparks lack documented success. This study demonstrates successful artificial rearing of toco toucan chicks using a homemade diet formulated from readily available ingredients (assorted fruits, chicken, mouse pups, vitamin-mineral supplements, and calcium supplements) to meet their nutritional requirements. While other studies have heavily relied on commercial diets (Seibels 1979; Gage and Duerr 2007; Leger et al. 2012), this homemade approach provides a viable, efficient and accessible alternative for institutions where such products are unavailable or prohibitively expensive.

These findings underscore the importance of adapting husbandry protocols to local resources and institutional needs, offering a replicable framework for other facilities engaged in the ex-situ management of this species. As part of the toco toucan conservation program, Parque de la Biodiversidad managed the intensive hand-rearing of the chicks until 60 days of age before transferring them to a partner institution, where they underwent a pre-release acclimatization period prior to soft release. Currently, the birds from this most recent cohort are in the pre-release stage, whereas individuals from the project's first year have already been released to reinforce the wild population.

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