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Comparison of glucose, serum iron and hemoglobin values in *Ateles geoffroyi* during dietary change

J. M. Sac Barrios[1*], *S. A. Morales*[2]

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ABSTRACT

Spider monkeys (*Ateles geoffroyi*) are considered frugivorous animals, their diet in the wild being composed mostly of fruits. In permanent or temporary captivity, the diets offered are usually high in commercial fruits and low in certain compounds, such as minerals, that build blood molecules. This study was conducted with a group of ten clinically healthy, transiently captive adult spider monkeys with whom a balancing and reformulation of the artificial diet offered was practiced. These animals were handled in the same way before, during and after the dietary change. They were captured prior to initiating the dietary change, twice during the modifications and one last time with the balanced diet already established. In these samplings, physical and biochemical parameters (glucose, iron, and hemoglobin) were evaluated. The main need of the original dietary balance was the decrease of excessive carbohydrates, and the increase of legumes and elements high in iron. In the evaluations performed during the diet change period, alterations in the biochemical parameters evaluated against the reference parameters were evidenced, including fluctuations in the glucose level, weight fluctuations, a significant increase in iron bioavailability and a fluctuation in hemoglobin levels that transiently increased together with the increase in the availability of dietary iron. It is concluded that, for each change in the diet offered in captivity to this species, at least two clinical evaluations, separated by 30 days, should be included to assess the clinical effects of the modification.

Keywords: spider monkey, frugivorous, temporary captivity, diet reformulation.

Comparación de valores de glucosa, hierro sérico y hemoglobina en *Ateles geoffroyi* durante un cambio de dieta

RESUMEN

Los monos araña (*Ateles geoffroyi*) se consideran animales frugívoros, y en vida libre su dieta se compone mayormente por frutos. En primates bajo cuidado permanente o temporal, las dietas ofrecidas suelen ser altas en frutas comerciales y bajas en ciertos compuestos, como minerales, que construyen moléculas sanguíneas. Este estudio se realizó con un grupo de diez monos araña adultos en cautiverio transitorio clínicamente sanos, con quienes se practicó balanceo y reformulación de la dieta artificial ofrecida.

[1] Universidad de San Carlos de Guatemala, Facultad de Medicina Veterinaria y Zootecnia. Ciudad de Guatemala, Guatemala.

[*] Correo electrónico: johana.sac.barrios@gmail.com

[2] Asociación Rescate y Conservación de Vida Silvestre, Centro de Rescate y Rehabilitación, Salud Animal, Aldea El Arrozal, Flores, Petén, Guatemala.

Estos se manejaron de la misma forma antes, durante y después del balanceo de dieta. Los monos fueron capturados antes de iniciar el cambio dietético, dos veces durante las modificaciones y una última con la dieta balanceada ya establecida. En estos muestreos, se evaluaron parámetros físicos, bioquímicos (glucosa, hierro y hemoglobina). La necesidad principal de balance dietario original fue la disminución de los carbohidratos excesivos y el incremento de legumbres y elementos altos en hierro. Durante las evaluaciones realizadas en el periodo de cambio de dieta se evidenciaron alteraciones en los parámetros bioquímicos evaluados frente a los parámetros de referencia, incluyendo fluctuaciones en el nivel de glucosa, fluctuaciones de peso, un incremento significativo en la biodisponibilidad de hierro y una fluctuación en los niveles de hemoglobina que incrementaron transitoriamente en conjunto al alza en la disponibilidad de hierro dietético. Se concluye que, para cada cambio en la dieta ofrecida en cautiverio a esta especie, se deben incluir al menos dos evaluaciones clínicas, separadas por treinta días, para evaluar los efectos clínicos de la modificación.

Palabras clave: mono araña, frugívoro, cautiverio transitorio, reformulación dietaria.

INTRODUCTION

The majority of ateline primates feed on an extensive variety of plants, primarily consuming edible fruits (Rosemberg & Strier 1989) and are described as specialist individuals in their consumption (Dew 2005). Food choice may be influenced by multiple factors related to the morphology of the fruits to be consumed, their quality and quantity, and their distribution (Stevenson & Link 2010). Both their frugivorous nature and their preference for sugars (and extreme sensitivity to them) associated with food (Laska *et al*. 1998) suggest that the content of soluble carbohydrates could be an important determinant in food choice.

The spider monkey is a primate mammal of the Atelidae family, genus *Ateles*, with a dietary preference for wild fruits, classified as a frugivorous animal (Vidal-García *et al*. 2016). In 2020, the species *Ateles geoffroyi* was evaluated for inclusion in the IUCN Red List of Threatened Species. It is classified as "endangered A4cd" (Cortés *et al*. 2021). Among the main threats to this species are habitat loss, indiscriminate logging of trees that provide them with food and the illegal trafficking of wild animals for the pet trade (Estrada *et al*. 2006; Ramos-Fernandez & Wallace 2008).

The natural diet of ateline primates in the wild consists of approximately 80% to 90% ripe fruits, with the remaining 10% to 20% comprising tender leaves, seeds, flowers, and even some insects. Spider monkeys (*Ateles* spp.) are considered energy maximizers (Rosenberger & Strier 1989) due to their high activity level, requiring the utilization of this type of energy (Pozo 2004; Felton *et al*. 2008) across vast territories, and because of their preference for consuming fruits rich in sugars and lipids (Di Fiore *et al.* 2008).

Wild animals, when removed from their natural environment for various reasons, undergo radical changes in several aspects of their lives, including feeding. Therefore, it is necessary to consider various factors when formulating a diet for these individuals under human care (Patiño 2019). The quality, acceptance, and adaptation to diets offered *ex situ* have a direct relationship with nutritional status, protein, and energy availability. Additionally, handling stress, sustained stress, and exposure to parasites and other physiological and pathological conditions can have direct effects on homeostasis, reflected in hematological and biochemical alterations (Ávila 2007). The best practice when choosing a diet is to broadly meet the nutritional requirements of the species in question, containing sufficient fiber for normal digestion and ingredients that stimulate individual behavior (Patiño 2019). The general nutritional requirement for an adult New World primate for maintenance, regardless of sex and gender, is 150-160 Kcal/kg body weight per day (Crissey et al. 1998), protein 15%, fat 4%, Ca 0.5%, P 0.4%, vitamin A 10,000 IU/kg, and vitamin D 2,000 IU/kg (National Research Council [NRC] 2003).

Carbohydrates are the most abundant compounds in plants, besides water. They are found in aerial parts (stems, leaves, flowers, fruits, and seeds) and underground parts; they constitute around 50%-80% of dry matter. Glucose, one of the important monosaccharides in animal nutrition, is present in fruits and some vegetables. Guyton (1989) details that when the body receives excessive amounts of carbohydrates, they can be immediately used for energy production or stored as glycogen or fat in adipose tissue.

Although glucose can be used for energy in all cells, it is essential for erythrocytes; its concentration in the blood reflects the nutritional conditions of the animal (Bush 1982). However, if it is not available in the diet or in the form of glycogen, it can be produced in small amounts through the gluconeogenesis pathway. Therefore, glucose in the short term is not considered an essential dietary element, but there are energy costs associated with gluconeogenesis, and it is likely that minimal concentrations of these should be present for optimal health and metabolic efficiency.

Minerals are critical for the health of a living organism. In animals, minerals play a vital role in the growth and maintenance of tissues as structural components of organs and tissues (Barboza *et al.*, 2008), as cofactors or activators in enzymatic systems, as well as components of body fluids (maintaining osmotic pressure, membrane permeability, among others), and in maintaining acid-base balance in the body (NRC 2003; Robbins 1993).

Trace elements required include iron. In wildlife, primates obtain these minerals from plants and animal tissues, depending on their feeding habits (Ruiz & Zambrano 2015). Green leaves, in general, are a good source of these elements. Primates under human care, regardless of their purpose, meet many of their mineral requirements through additions of specific minerals to diets containing ingredients that would otherwise be nutritionally incomplete. Browsing (fresh or dry foliage) offered to primates under human care can also contribute to the dietary supply of essential minerals (NRC 2003).

Numerous studies have recognized the utility of hematology and serum chemistry as tools in the management and conservation of wildlife, including monitoring during diet changes (Ávila 2007). Blood plays a direct or indirect role in most biochemical processes in the organism, and the alteration of these processes helps in the detection of deficiencies (Roca *et al.* 2003).

Biochemical tests are employed to evaluate different nutritional imbalances, as achieving an adequate nutritional status will depend on both the health status and productive performance of individuals.

Various factors affect nutritional status, but these can be assessed through these analyses (Figueroa 2004). Thus, throughout this study, spider monkeys in rehabilitation are evaluated in terms of dental status, hemogram, glucose, and serum iron, assessing their physiological status during the transition of the dietary modification formulated based on their nutritional requirements and whether the changes in diet formulation meet these requirements.

Fluctuations in hematological and biochemical values are expected with any dietary changes; this phenomenon is not exclusive to New World primates. To our knowledge, no prior research in this species has examined changes in glucose, iron, and hemoglobin during or after dietary adjustments.

MATERIALS AND METHODS

Study area

The present study was conducted at the facilities of the Wildlife Rescue and Rehabilitation Center of the Wildlife Rescue and Rehabilitation Association (Arcas, for its acronym in Spanish), located in El Arrozal village, Flores municipality, Petén department, Guatemala.

Methods

The population selected for this study consisted of ten individuals of black-handed spider monkey (*Ateles geoffroyi*), seven females and three males at various stages of the rehabilitation process. The monkeys' ages ranged from three to six years old. Each one was identified through microchip and photographs. Four combined captures, manual and chemical restraint, were carried out on day 15, day 30, day 45, and day 75. The following procedures were conducted at each capture: first, general physical examination including determination of body condition on a scale of 1 to 5 (1 being emaciated and 5 obese), weighing, evaluation of the integumentary and locomotor systems, as well as oral cavity assessment, and secondly, blood sample collection.

Biometric data were recorded in clinical charts, including glucose results, complete blood count (red and white series), observations of physical condition, oral cavity condition, as well as photographs of the teeth and gums in frontal and lateral views. Evaluation of the oral cavity required a direct light source on the teeth and gloves. For blood extraction, tubes with EDTA anticoagulant, 3ml syringes, 23" needles, alcohol, cotton, and gloves were used. The sample was obtained through sterile puncture of the femoral vein. Blood glucose measurement was performed using a glucometer (ACCU-CHEK®). Hemogram results from an automated five-part analyzer (Abaxis, Vetscan, HM5), glucose, and serum iron were attached alongside the clinical chart. The blood hemogram and biochemistry were interpreted using values provided in the International Species Information Systems (2013).

Diets offered in this study:

- Diet 1, consisting of 863 kilocalories, 163 total digestible carbohydrates, and 17% protein.
- Diet 2, the reformulated diet based on nutritional requirements according to the NRC, consisted of 504 kilocalories, 72 total digestible carbohydrates, and 25.27% protein.

To determine the statistical significance of the proposed dietary change effect in this article, the Chi-square test was used. This and all graphs were created using the R program, version 4.2.0 (2022-04-22 ucrt).

This study was conducted following the animal welfare and bioethics guidelines of the Global Federation of Animal Sanctuaries (GFAS), ensuring that no individuals were harmed during the process.

RESULTS

During each capture, blood glucose, iron, and hemoglobin levels were assessed. While hemoglobin and iron were measured in all samples, glucose was measured in only three out of the four samplings due to equipment failures. Each element was individually evaluated and analyzed at the conclusion of the study to identify any relationships among them.

In the glucose measurement, it was observed that, during the first two events, the ranges were similar but the mean in the second sampling, with results of 79.6 mg/dl, decreased compared to the first of 82.6 mg/dl. In the last sampling, an increase in the range amplitude and the mean of glucose values was observed, resulting in 92.1 mg/dl, as shown in figure 1.

The means of iron from the first and second samplings were similar as both ranged between 79.3 pg/dl and 81.4 pg/dl; these levels were significantly lower than those shown in the third sampling, 101.4 pg/dl; and the fourth sampling, 96 pg/dl. During the third event, both the mean and the range increased compared to the other events. The ranges were different in the last sampling, with the third sampling showing the widest amplitude.

In the measurement of hemoglobin, it was found that during the first and second sampling, the ranges were similar, but the mean of the second sampling decreased compared to the first. In the fourth sample,

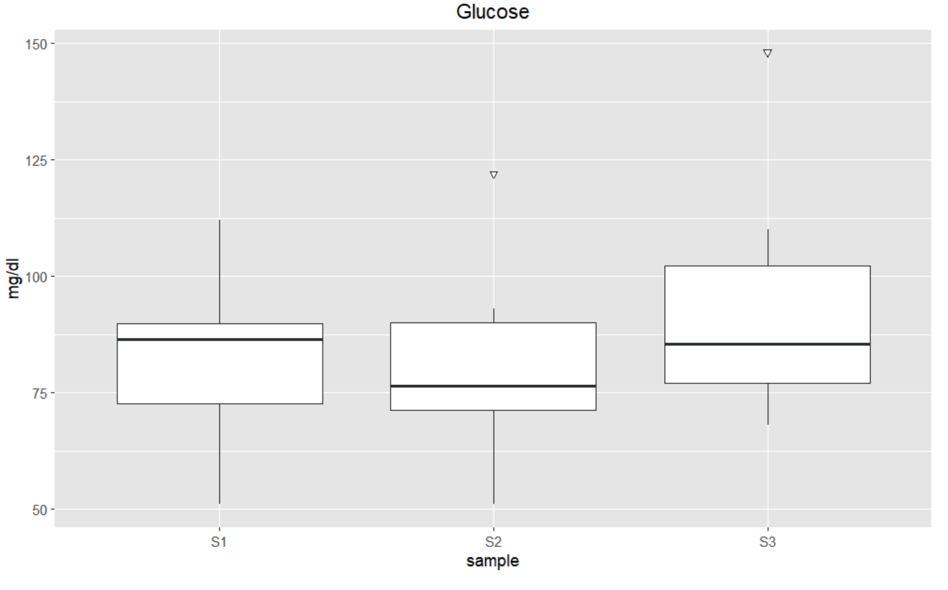

FIGURE 1. Three glucose sampling sessions in a group of 10 black-handed spider monkeys (*A. geoffroyi*). Source: own elaboration.

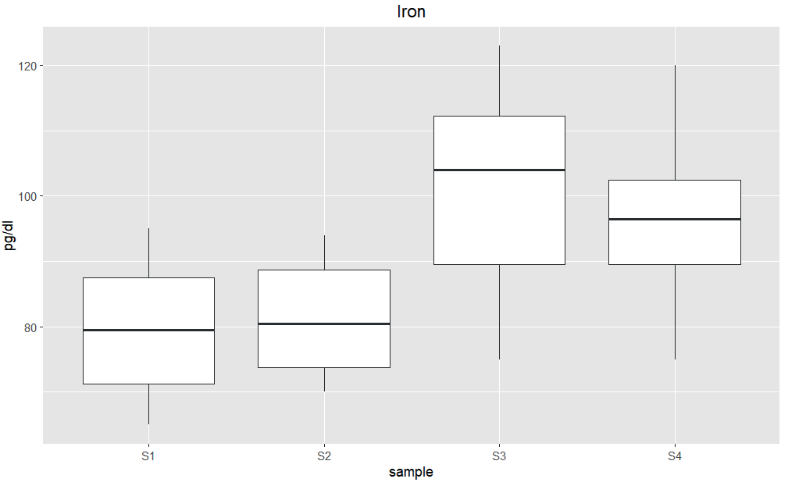

FIGURE 2. Four iron sampling sessions in a group of 10 black-handed spider monkeys (*A. geoffroyi*).
Source: own elaboration.

an increase in the range amplitude of this element was observed, as well as the mean; with the range represented on the y-axis and the sampling number on the x-axis as depicted in figure 3.

The weighing of the group of spider monkeys at the beginning of the study shows an overall average of 4.73 kg within a described range of 3 to 6.3 kg. The average shown at the end of the study is

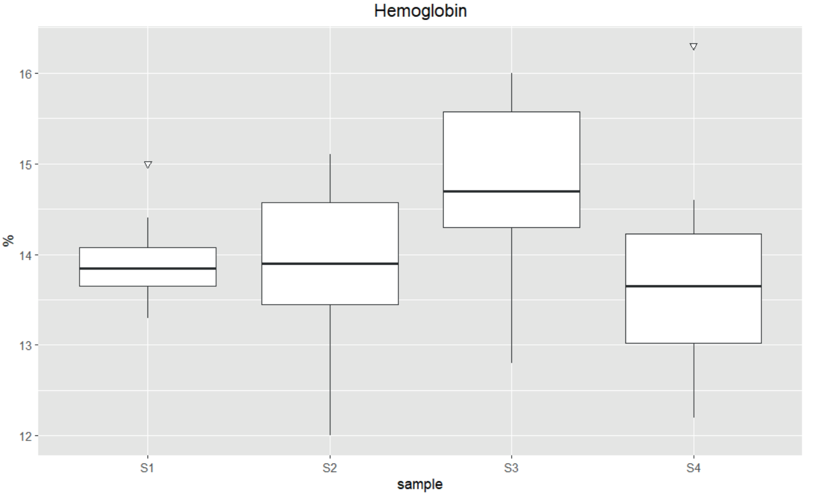

FIGURE 3. Four hemoglobin sampling sessions in a group of 10 black-handed spider monkeys (*A. geoffroyi*).
Source: own elaboration.

5.32 kg, within a range of 4.1 to 6.9 kg, indicating a weight gain obtained at the end based on the balanced diet offered, as indicated in table 1.

TABLE 1. Initial and final weights of a group of 10 black-handed spider monkeys (*A. geoffroyi*) during the diet change

Weight in kg		
	Beginning	**Ending**
ID 965000000300831	4	4.3
ID 965000000301005	5.1	5.7
ID 965000000301258	4.4	4.1
ID 965000000355029	5.3	5.9
ID 965000000354878	3.7	4.6
ID 968000010056626	3	4.6
ID 965000000301059	6.3	6.9
ID 965000000300599	5	6.3
ID 965000000300295	4.7	5
ID 965000000355788	5.8	5.8
Average	4.73	5.32

Source: own elaboration.

Body condition, as determined by Russel (1984), sets a five-point scale and observations on four body areas and is considered an appropriate system for diagnostic, prognostic, and decision-making purposes. Therefore, the condition of each individual ranges from 2 to 3, with a general average of 2.75 at the beginning of the study. By the end of the study, the range narrows to 2.5 to 3, with a general average of 2.95.

TABLE 2. Initial and final body condition of a group of 10 black-handed spider monkeys (*A. geoffreyi*) during the diet change

Body condition (scale from 1 to 5)		
	Beginning	**Ending**
ID 965000000300831	3	3
ID 965000000301005	3	3
ID 965000000301258	3	3
ID 965000000355029	2	3
ID 965000000354878	2	2.5
ID 968000010056626	2.5	3
ID 965000000301059	3	3
ID 965000000300599	3	3
ID 965000000300295	3	3
ID 965000000355788	3	3
Average	2.75	2.95

Source: own elaboration.

DISCUSSION

The primary product of digestion in monogastric animals is glucose, allowing them to use it as the main source of energy. By modifying the food rations that provide this nutrient, during the initial sampling and subsequent ones, blood glucose levels were affected, resulting in a series of variations in the measurements obtained at each capture, influenced by the nutrient availability in the diet (Felton *et al*. 2009) and the quantity ingested by the individual. The measurements conducted in this study demonstrate changes influenced by a transient relative decrease when implementing the diet change and a final increase with the balanced diet in place.

The results obtained from the glucose level measurements in the spider monkeys of this study demonstrated different range values and means in each of the samplings; wherein, during the first two events, similar ranges were obtained, suggesting a period of metabolic adaptation to changes in the diet. These fluctuations may also be affected by alterations in appetite levels, which may even be of hormonal origin, and if present, would influence the stress mechanism and energy homeostasis, in addition to alterations in nutrient metabolism and subjective appetite (Greenway 2015). Apart from individual experience, the acceptance of new foods may vary with age and species (Prescott *et al*. 2005). However, in the last sampling, the ranges and mean were higher as they maximized the energy obtained from the foods offered, thus returning to normal ranges; this is an adaptive strategy that influences their consumption behavior (Markle et al. 2015).

The monkeys in this study exhibited good body condition and appropriate weight for their age; however, spider monkeys are relatively large animals, and there is a relationship between body size and predisposition to hypoglycemia. Likewise, the stress caused by the dietary modification, which involved a 16% decrease in fruit provision and its consequent decrease in availability, may be the reasons for the transient drop in glucose levels in the second measurement. This, with a mean of 79.6 mg/dl, was found to be 3 points lower compared to the mean of the first sampling.

By successfully meeting the energy requirements of the monkeys through the intake of various nutrients offered in the diet ingredients during a reduced or absent stress period and adaptation to the modified diet, it was observed that in the last sampling, blood glucose levels increased again, with a range of 68-148 mg/dl and a mean of 92.1 mg/dl.

Similarly to glucose, the absorption of non-heme iron is strongly influenced by the diet composition, due to the concomitant effect between the ingested foods and the absorbed elements now available in the organism (González Urrutia 2005). Factors affecting bioavailability and absorption include the chemical form of iron and the levels of other components in the diet (AZA Prosimian Taxon Advisory Group [PTAG] 2003). This type of iron is present in plant-derived products. By including foods with a high iron content such as celery, lettuce, broccoli, cauliflower, iron absorption by enterocytes increases, resulting in a rise in iron levels. Iron levels, like those of hemoglobin, exhibited a similar variation during the change process in the first and second events due to unfamiliarity with the new ingredients. However, during the last two samplings, an increase in ranges and their respective means was observed, due to greater acceptance of these ingredients reflecting adequate integration into the diet and consequent intake.

The behavior of iron remained within the same values in the first and second samplings; this is due to the low acceptance observed at the beginning of the new diet, with values remaining within the same range; however, this changed for the third sampling as the intake of the vegetables providing non-heme iron increased. In the last sampling, the ranges and mean decreased by 20% compared to the third sampling, but still remained higher compared to the first two events.

Iron can be found in ferrous form (Fe2+), which donates electrons, or in ferric form (Fe3+), which receives them. This ability of iron makes it a useful component in cytochromes and oxygen-carrying molecules (myoglobin and hemoglobin). Therefore, increasing iron absorption results in greater availability of this element, causing hemoglobin levels to increase or decrease in proportion to iron levels in the diet. This is evidenced by the maintenance of hemoglobin levels during the first two samplings, the increase during the third sampling, and the partial decrease in the last sampling.

Iron is conserved and continuously recycled by the body due to the absence of an excretory mechanism (Follerat 2016). Iron levels during the fourth sampling decreased, although not drastically, reaching stable levels within the established ranges for the specimens. This is explained by the fact that once the necessary iron to form the heme group of hemoglobin is obtained, the remaining mineral is stored in hepatocytes and macrophages (Follerat 2016). Therefore, a moderate decrease in hemoglobin levels to normal levels during the last sampling may be due to a decrease in the need for iron, as it meets the baseline requirements.

According to Burkholder (2000), body condition is a subjective assessment of the amount of energy stored as fat and muscle, which is an important part of individual evaluation. This parameter helps determine if individuals are within an acceptable range (2.5 to 3 out of 5 points), indicating good health status and no visible prevalence of diseases throughout the study period. The overall average was 2.75 at the beginning and 2.95 at the end, so the increase in this score indicates a favorable response to the dietary modification.

CONCLUSION

The provision of balanced diets to wild animals under human care is essential for proper development and maintenance of health status. These modifications depend on the species and nutritional requirements. Therefore, these changes may lead to significant transient metabolic changes. After a period of behavioral and metabolic adaptation, normal hematological values reappear within the established ranges evaluated. These transient fluctuations, although evident, are not permanent and can be considered a normal metabolic episode.

Glucose levels within the organism varied according to the presented stress and the proportion of fruits offered and ingested. Subsequently, normoglycemic levels were reached, achieving satisfactory adaptation to the modifications of the new diet. An increase in serum iron values was evidenced, starting from day 45, as well as a significant increase in hemoglobin concentration, followed by a decrease and return to values within the reference range for the species. It is assumed that this variability responds to the ferric bioavailability generated by the concentration of inorganic iron in the foods offered in the balanced diet.

Both body condition and weight were key indicators for the physical evaluation and nutritional status of the individual. Body condition showed maintenance or improvement at each capture, making these tools highly valuable in the management of spider monkey specimens, as they can be performed remotely and without causing stress, especially when direct physical examination or hematological analysis is not possible.

When making a dietary modification, if the intake is adequate, animals do not suffer from continuous stress or exposure to pathogens. Additionally, changes in hematological values of glucose, iron, and hemoglobin should not be considered valid in less than 75 days after the definitive change in diet.

ETHICS COMMITTEE

The tests were conducted with the intention of strengthening the health protocols of the institution. No experimentation requiring the involvement of a different collegiate body was conducted. Additionally, Arcas holds the necessary authorizations and certifications for animal handling to conduct the research. The rescue center is legally constituted, and its management protocols are authorized by the highest wildlife authority in Guatemala, the National Council of Protected Areas (Conap, for its acronym in Spanish). Furthermore, Arcas is accredited by GFAS for excellence in wildlife management and care.

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USE OF ARTIFICIAL INTELLIGENCE

No artificial intelligence programs were used for this study.

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